INFLUENCE OF pH ON THE SURFACE HARDNESS AND MICROSTRUCTURE OF MINERAL TRIOXIDE AGGREGATE AND BIOCEM - AN INVITRO STUDY

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CERTIFICATE

This is to certify that this dissertation titled **"INFLUENCE OF pH ON THE SURFACE HARDNESS AND MICROSTRUCTURE OF MINERAL TRIOXIDE AGGREGATE AND BIOCEM - AN INVITRO STUDY"** is a bonafide record of work done by **D.NIRMALA** under our guidance during the study period between **2007-2010.**

This dissertation is submitted to THE TAMIL NADU Dr. M.G.R. MEDICAL UNVERSITY, in partial fulfillment for the degree of MASTER OF DENTAL SURGERY – CONSERVATIVE DENTISTRY AND ENDODONTICS, BRANCH III. It has not been submitted (partial or full) for the award of any other degree or diploma.

Guided by:

Dr. M.Rajasekaran, M.D.S.,
Associate Professor,
Department of Conservative Dentistry and Endodontics,
Ragas Dental College and Hospital, Chennai

Dr. R. Indira, M.D.S.,
Professor and H.O.D,
Department of Conservative Dentistry and Endodontics,
Ragas Dental College and Hospital, Chennai

Dr. S. Ramachandran, M.D.S.,
Professor and Principal,
Department of Conservative Dentistry and Endodontics
Ragas Dental College and Hospital, Chennai

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CONTENTS

Introduction

INTRODUCTION

Apicoectomy followed by retrograde filling is a well established procedure to treat teeth with persistent periapical infections and teeth in which conventional root canal therapy has failed.[3] Many materials have been advocated for use as root-end fillings, but none have proven to consistently provide a fluid tight seal.[50]

Mineral trioxide aggregate (MTA) was developed and introduced for use in endodontics for the repair of root perforations at Loma Linda university in the 1990's.[9] It was then widely used as a root-end filling material and for vital pulp therapy, including direct pulp capping and pulpotomy of immature teeth with vital pulps (apexogenesis). In addition, because of its sealing ability, it was also suggested as an apical barrier in treatment of teeth with open apices and necrotic pulps (apexification).[31]

The use of MTA as a root end filling material was identified because the material is a hydraulic cement that sets in the presence of water.[9] MTA is essentially Portland cement with 4:1 proportions of

bismuth oxide added for radioopacity. Camilleri et al has shown using x-ray diffraction analysis that MTA is composed primarily of tricalcium silicate, dicalcium silicate and bismuth oxide, which on hydration produce a silicate hydrate gel and calcium hydroxide, thus rendering the material biocompatible.[8]

Two commercial forms of MTA are available. ProRoot MTA (Dentsply), is available as grey and white MTA, both with similar chemical and physical properties. MTA-Angelus has also become available in the market.[9] The more esthetic white–colored preparation lacks the tetra calcium alumino ferrite. The lack of this iron - containing compound may account for its white appearance.[42]

Hydration of MTA occurs in two stages. The initial reaction between tricalcium aluminate and water in the presence of calcium sulphate results in the production of ettringite. Tricalcium and dicalcium silicate react with water to produce calcium silicate hydrate and calcium hydroxide, which is leached out of the cement with time.[13]

The physicochemical basis of the biological properties of MTA had recently been attributed to the production of hydroxyapatite,

when the calcium ions released by the MTA comes into contact with tissue fluid. The hydration rate is characteristic of the progress of cement setting. Scanning electron microscopy (SEM) can be used to quantify the observable porosity as an indicator of cement hydration. Sufficient water is required during the setting of the cement to ensure a comprehensive hydration reaction.[39]

In some clinical situations MTA may be directly exposed to an acidic environment which can affect the physical and chemical properties of MTA placed in that area. MTA has a pH of 10.2 initially and which can increase to 12.5 within three hours after mixing. However, it is possible that variations in the pH of the host tissues can occur due to the presence of pre-existing pathological conditions, like open apex, non vital teeth with periapical lesions, lateral or furcal perforations with radiolucent lesions.[31]

The present study was designed to evaluate the surface microhardness of white and grey MTA following exposure to a range of acidic environments during hydration. In addition, an indigenous experimental cement BIOCEM has also been studied. The

morphological and microstructural features of the samples were studied using SEM.

AIM:

The aim of this study was to evaluate the influence of pH on the surface hardness and microstructure of Mineral trioxide aggregate and an experimental cement, Biocem.

OBJECTIVE:

1. The objective was to measure the surface microhardness of mineral trioxide aggregate and Biocem, using Vicker's microhardness testing machine
2. To study the microstructure of mineral trioxide aggregate and Biocem under scanning electron microscope
3. To compare the microhardness and the microstructure of the grey, white Mineral trioxide aggregate with an experimental cement Biocem.

Review of literature

REVIEW OF LITERATURE

Seltzer et al (1985)[41] explored the possible etiological factors of flare-ups during or after endodontic therapy. Although the reasons for such exacerbations are not clear, a number of hypothesis are discussed. Among these the important factors are a) alteration of the local adaptation syndrome b) changes in periapical tissue pressure c) microbial factors d) effects of chemical mediators e) changes in cyclic nucleotides f) immunological phenomenon g) various psychological factors

Torabinejad et al (1993)[45] evaluated the sealing ability of amalgam, super EBA, and a mineral trioxide aggregate when used as root end filling materials, using rhodamine B fluorescent dye and confocal microscope. Thirty single canal teeth were cleaned, shape, and obturated with gutta-percha and root canal sealer. After application of nail polish to the internal surface, the apical 3mm of each root was resected and 3 mm deep root end preparations were made. The roots were randomly divided into 3 groups and root end preparations filled with the experimental material. All roots were exposed to rhodamine B, Fluorescent dye for 24 hrs and the penetration was measured using

confocal microscope. Analysis showed that mineral trioxide aggregate leaked significantly less than amalgam and super EBA.

Lee et al (1993)[25] compared the sealing ability of the MTA with that of amalgam and IRM in experimentally induced lateral perforation in extracted human teeth. Fifty sound, extracted mandibular and maxillary molars were used. A perforation was created on the mesial root surface at about a 45 degree angle to the long axis of each tooth. The tooth was then placed into a saline soaked 'Oasis' to simulate a clinical condition. After placing the repair material into the perforations, the teeth were kept for 4 weeks in the oasis model. The perforation sites were then stained with methylene blue for 48 hours, sectioned and examined under a dissecting microscope. Results showed that the mineral tri oxide aggregate had significantly less leakage than IRM or amalgam ($p<0.05$) The mineral trioxide aggregate also showed the least overfilling tendency while IRM showed the least under filling tendency.

Torabinejad et al (1995)[44] conducted a study to determine the chemical composition, pH of the setting cement and radio opacity of MTA, and to compare the setting time, compressive strength, and

solubility of this material with those of the commonly used root end filling materials, amalgam, super EBA, and IRM. X-ray energy spectrometer in conjunction with scanning electron microscope were used to determine the composition of MTA, and the pH value of MTA was assessed with a pH meter using a temperature – compensated electrode. The radiopacity of MTA was determined according to the method described by the international organization for standardization. The results showed that the main molecules present in MTA are calcium and phosphorous ions. In addition, MTA has a pH of 10.2 initially, which rises to 12.5 three hours after mixing. MTA is more radiopaque than super EBA and IRM. Amalgam had the shortest setting time (4min) and MTA the longest (2h 45 min). At 24 hrs, MTA had the lowest compressive strength (40 Mpa) among the materials, but it increased after 21 days to 67 Mpa. Finally, except for IRM, none of the materials, tested showed any solubility under the conditions of this study.

Pitt Ford et al (1995)[34] evaluated histologically the tissue response to experimentally induced furcal perforations, repaired with amalgam or MTA either immediately or after salivary contamination. Intentional perforations made in the furcations of 28 mandibular premolars in 7

dogs were investigated histologically. In half the teeth, the perforation were repaired immediately with either amalgam or MTA. In rest perforation were left open to salivary contamination before repair. All repaired perforations were left for 4 month before histologic examination of vertical section through the site. Histologic evidence has shown that MTA has potential as a material for immediate repair of furcal perforation. In particular, 5 of 6 teeth had some cemental repair over the material. Study concluded that MTA is a far more suitable material than amalgam for perforation repair.

Bates et al (1996)[5] evaluated the ability of mineral trioxide aggregate (MTA) to seal the root end effectively. Seventy-six single-rooted, extracted human teeth were cleaned and shaped using a step-back technique. After root-end resection and ultrasonic preparation, 76 root sections were randomly allocated to three groups and filled with dental amalgam and cavity liner, Super-EBA, or MTA. Micro-leakage was assessed at 24h, 72h, 2wk, 4wk, 8wk and 12wk, using a fluid filtration measurement system. MTA was determined to be superior to amalgam, and comparable with Super-EBA in preventing microleakage when used as a root-end filling.

Yatsushiro et al (1998)[50] conducted an in-vitro study to evaluate the microleakage of MTA and a high copper admix amalgam in root-end preparations of 33 extracted , single rooted teeth, using a fluid conductive device. 3 mm deep class 1 cavity preparations were made on the resected root-end and filled with either MTA or amalgam. In the fluid conductive device , the root canals were filled with phosphate – buffered saline solution at a pressure of 10 psi. The flow of fluid was measured and compared at 1,2,3,4,8,12,16,20 and 24 weeks. The results showed MTA to have significantly lower microleakage after four weeks and lower variability compared with the amalgam group.

Fisher et al (1998)[19] conducted the study to determine bacterial leakage of MTA, compared with commonly used root – end filling materials. Fifty six, single rooted extracted human teeth were cleaned and shaped with a series of 0.04 taper rotary instruments. The canals were prepared, ends were resected, and cavities were made with ultrasonics to a depth of 3 mm using an aseptic technique. The root end cavities were filled with amalgam, IRM, super – EBA and MTA. The root ends were placed into 12 ml vials of phenol red broth using micro pipette, a tenth of mm of S. marcescens was placed into the

root canal of each tooth. Results showed that samples filled with zinc free – amalgam leaked bacteria in 10 to 63 days. IRM began leaking 28 to 91 days. Super EBA,42 to 101 days. MTA did not leak until 49 days. Analysis of the data indicates MTA to be the most effective root – end filling material.

Wu et al (1998)[49] measured the leakage of a few root – end filling materials in a longitudinal manner during a 1 year period, using a fluid – transport model. Hundred standard bovine root sections, each 3 mm high and with a central pulp lumen of 2.6 mm in diameter, were filled with 5 commonly used or potential root end filling materials. Leakage along these filling materials was determined using a fluid transport model at 24 hrs or at 3, 6 or 12 months interval. During the first 3 months, the percentage of leakage increased noticeably for amalgam and super EBA, whereas it decreased noticeably for mineral trioxide aggregate. The increased leakage of amalgam and super EBA decreased with time, whereas the improved seal of MTA was maintained until the end of the experiment.

Nakata et al (1998)[30] evaluated the ability of mineral trioxide aggregate (MTA) and amalgam to seal furcal perforation in extracted human molars using an anaerobic bacterial leakage model. Perforations were made using high speed bur in 39 molars. The teeth were randomly divided into two experimental groups of 18, with the remaining 3 teeth used as positive control. Experimental group 1 was prepared with MTA and Group 2 with amalgam. 3 additional teeth without perforation served as negative control. A dual chamber anaerobic bacterial leakage model was assembled .Brain heart infusion broth with yeast extract, hemin, menadione, and the chromogenic indicator bromocresol purple was used as the culture broth fusobacterium nucleatum. Eight of 18 amalgam samples leaked. MTA was significantly better than amalgam in preventing leakage of F.nucleatum past furcal perforation repairs.

Torabinejad et al (1999)[43] investigated an experimental material, mineral trioxide aggregate (MTA), as a potential alternative restorative material to the presently used materials in endodontics. Several in vitro and in vivo studies has been highlighted in this article and they showed that MTA prevents microleakage, is biocompatible, and promotes regeneration of the original tissues when it is placed in

contact with the dental pulp or periradicular tissues. This article describes the clinical procedures for application of MTA in capping of pulps with reversible pulpitis, apexification, repair of root perforations nonsurgically and surgically, as well as its use as a root-end filling material.

Aqrabawi et al (2000)[3] compared apical microleakage of MTA following reverse retrograde root filling with that following amalgam and EBA retrofilling. Root canals of 79 extracted teeth were instrumented and obturated with vertically condensed gutta percha. Apical resection to 3mm depth was made. Teeth were divided into 3 groups. First group with amalgam, second group with EBA and third group with MTA was retrofilled. Dye leakage was evaluated by immersion in 1% methylene blue for 72 hours.56% of the group filled with amalgam and 20% of the group filled with EBA showed dye leakage beyond the retrofilling material whereas the MTA group showed none. In conclusion, MTA cement provides a better seal than amalgam and EBA cement when used as retrograde filling material.

Roy et al (2001)[35] One hundred fifty – six single rooted human extracted teeth received root canal treatment, apicoectomy and

ultrasonic root end preparations. The roots were divided into six groups of 24 teeth. The groups were filled with amalgam, geristore, super–EBA, mineral trioxide aggregate (MTA), calcium phosphate cement(CPC), or MTA with CPC matrix, respectively. Immediately after root end filling, 12 teeth from each group were exposed to a pH of 5.0 for 24 hours and 12 teeth were exposed to a pH of 7.4 for 24hrs.Twelve teeth served as controls. All teeth were exposed to pelican ink for 5 days and cleared. Linear dye leakage was recorded. Data were statistically analysed. An acidic pH significantly reduced leakage of geristore and MTA with CPC matrix, whereas leakage of all the other materials was not affected by pH. In conclusion an acidic environment did not hinder the sealing ability of any materials tested, and enhanced the sealing ability of geristore and MTA with CPC matrix.

***Schmitt et al (2001)*[40]** reviewed the multifaceted use of ProRoot MTA root canal repair material.MTA has been reported to have superior biocompatibility and sealing ability and is less cytotoxic than other materials currently used in pulpal therapy. He reviewed MTA's physical and biological properties and the clinical techniques of direct

pulp capping, apexification and repair of failed calcium hydroxide therapy.

Andelin et al (2002)[2] used Forty-six single rooted extracted human teeth in his study. After cleaning and shaping, twenty canals were randomly selected for obturation with MTA placed in an orthograde manner (Group 1). Another twenty roots were obturated with gutta-percha and Kerr EWT sealer, using warm vertical compaction (Group 2). The apical 3mm of each root in both groups were resected at approximately 45 degree to its long axis. The teeth were aged for 48h in a humidor. Based on the results, it appears that the resection of set MTA does not affect its sealing ability.

Fridland et al (2003)[20] determined the solubility and porosity of MTA when mixed with different water to powder proportions and to establish the chemical composition of the salts dissolved from the mixture. Four sets of specimens using the following water to powder proportions were prepared - 0.26, 0.28, 0.30 and 0.33 grams of water per gram of cement. It was determined that the degree of solubility and porosity increased as the water to powder ratio increased. This study found that the water that had been in contact with the

specimens had an alkaline pH between 11.94 and 11.99. The calcium found in the solution should be in its hydroxide state at this high pH level. This ability to release calcium hydroxide could be of clinical significance because it could be related to the proven capacity of MTA to induce mineralization.

Aminoshariae et al (2003)[1] evaluated how well MTA adapts to the walls of simulated root-canal system of varying lengths using radiographic and microscopic techniques and when placed by hand and ultrasonic methods. Eighty polyethylene tubes were divided into four groups of 20 tubes each. The tubes were prepared to receive, 3, 5.7 and 10 mm length of MTA respectively 10 samples of each lengths had MTA placed and condensed by the hand method and the other 10 by the ultrasonic method. After condensation, the samples were evaluated with a light microscope and radiographs for the degree adaptation of the MTA to the tube walls and for the presence of voids. Results demonstrated hand condensation resulted in better adaptation to the tube walls and less voids than the ultrasonic method.

Costa junior et al (2003)[14] studied tissue reactions to a component of root canal system bacteria. Lipoteichoic acid (LTA) , present in gram

- positive microorganisms, has physiochemical characteristics that allow it to act as an immunogen. Due to polymicrobial characteristics of root canal infections, LTA can participate in the development of periapical disease. He studied the reaction of rat subcutaneous tissue to Teflon fibre implants, filled with fibrinol soaked in lipoteichoic acid. He found that LTA provoked an inflammatory tissue reaction.

***Duarte et al (2003)*[17]** evaluated the pH and calcium ion release of 2 materials used for root -end fillings and perforation repair. ProRoot and MTA- Angelus were placed in plastic tubes and immersed in glass flasks containing deionized water. After 3,24,72 and 168 hrs ,the water in which each had been immersed was tested to determine the pH changes and the calcium released. He found that the values for pH and calcium ion release were slightly higher for MTA –Angelus than ProRoot. The results suggested that both materials release calcium and promote alkaline pH.

***Lee et al (2004)*[26]** evaluated how various physiological environments affect the hydration behavior and physical properties of mineral trioxide aggregate (MTA) using scanning electron microscope, X-ray diffraction (XRD) and microhardness tests. They found that the

microstructure of hydrated MTA consists of cubic and needle-like crystals. The former comprised the principal structure of MTA, whereas the later were less prominent and formed in the inter-grain spaces between the cubic crystals. MTA samples were hydrated in distilled water, normal saline, pH 7, and pH 5. However, no needle-like crystals were observed in the pH 5 specimens, and erosion of the cubic crystal surfaces was noted. XRD indicated a peak corresponding to Portlandite, a hydration product of MTA, and the peak decreased noticeably in the pH 5 group. The pH 5 specimens' microhardness was also significantly weaker compared to the other three groups (p<0.0001). These findings suggest that physiological environmental effects on MTA formation are determined, in part, by environmental pH and the presence of ions. They showed that an acidic environment of pH 5 adversely affected both the physical properties and the hydration behavior of MTA.

Menezes et al (2004)[29] investigated the pulpal responses of dog's teeth after pulpotomy and direct pulp protection, with MTA- Angelus, Pro- Root MTA, Portland cement. Seventy six teeth were treated with these materials. One hundred and twenty six days after treatment, the animals were sacrificed and the specimens removed and prepared for

histological analysis .He found that all the materials demonstrated similar results when used as pulp capping materials .Pulp vitality was maintained in all specimens and the pulp had healed with a hard tissue bridge. He concluded that the materials used in this study were equally effective as pulp protection materials following pulpotomy.

Camilleri et al (2004)[7] examined the biocompatibility of two commercial forms of mineral trioxide aggregate (MTA), by evaluating the morphology of an established cell line. The two cements were cast on glass cover slips and cured for 1 or 28 days. Saos-2 osteosarcoma cells were trypsinized and seeded at a density of 1×10^{5} cells and were then placed in medium over the material-coated coverslips for 1, 5 and 7 days. Cell morphological investigation was performed by scanning electron microscopy at various magnifications ranging from 250 to 500x . Results showed that the 1-day cured samples of two commercial forms of MTA showed good biocompatibility. However, the 28 days cured samples were less biocompatible after 1 and 5 days.

Main et al (2004)[27] evaluated the success rate of root perforation repairs using MTA. Sixteen cases were included that met the criteria

for the study. Pre treatment, immediate post treatment and atleast 1 year follow –up radiographs were evaluated in a double blind manner to determine the presence or absence of any pathologic changes adjacent to the perforation site. The results showed that all 16 cases demonstrated normal tissue architecture adjacent to the repair site at the recall visit. Teeth with existing lesions showed resolution of the lesion, and teeth without preoperative lesions continued to demonstrate absence of lesion formation at the follow-up visit. The result proves that, MTA provides an effective seal of root perforations and shows promise in improving the prognosis of perforated teeth that would otherwise be compromised.

Ferris et al (2004)[18] compared the ability of two types of MTA for sealing furcation perforations in human molars using an anaerobic leakage model. Forty human maxillary and mandibular molars were randomly divided into experimental groups of 10, with 2 teeth used as positive controls and 2 teeth without perforations used as negative controls. Group 1 was repaired with gray coloured MTA and group 2 with off white coloured MTA. Results showed that there was no significant difference between the gray type of MTA and the white type of MTA in allowing the passage of anaerobic organisms .

Matt et al (2004)[28] investigated the use of mineral trioxide aggregate (MTA) as an apical barrier by comparing the sealing ability and set hardness of white and gray MTA. Forty four root segment were prepared to simulate an open apex. Apical barriers of white and gray MTA were placed to a thickness of 2mm or 5 mm. The samples were obturated immediately (1 step) or after the MTA set for 24 h (2 steps). After placement in methylene blue dye for 48h, the samples were sectioned for leakage analysis and micro hardness testing of barriers. Results suggested that a 5mm apical barrier of gray MTA, using 2 steps produced the best apical barrier.

Dammaschke et al (2005)[15] completely analysed the chemical composition of ProRoot MTA in comparison to two commercially available Portland Cements. Another goal of this study was to investigate the bonding reactions by X-ray photon electro spectroscopic (XPS) surface analysis and give an insight into the structural properties by analyzing the morphological and physical properties of both ProRoot MTA and Portland cements. Results showed that, in ProRoot MTA, the amount of gypsum is approximately half of that of the portland cements. ProRoot MTA

consists of less toxic heavy metals (Cu, Mn, Sr) less chromophores (Fe 3+) and less Al-species, but contains about 2 % of Bi. The Portland cements are composed of particles with a wide range of size, whereas ProRoot MTA showed a uniform and smaller particle size.

Camilleri et al (2005)[8] evaluated the biocompatibility of mineral trioxide aggregate and accelerated Portland cement and their eluants by assessing cell metabolic function and proliferation. The chemical constitutions of gray and white portland cement, grey and white MTA and accelerated portland cement produced by excluding gypsum from the manufacturing process (Aalborg white) was determined using both energy dispersive analysis and X-ray diffraction analysis. Biocompatibility of the materials was assessed using a direct test method were cell proliferation was measured quantitatively using Alamar Blue TM dye and an indirect test method where cells were grown on material elutions and cell proliferation was assessed using methyltetrazolium assay. Biocompatibility testing of the cement eluants showed the presence of no toxic leachables from the grey or white MTA, and that the addition of bismuth oxide to the accelerated Portland cement did not interfere with biocompatibility. The elution

made up of calcium hydroxide produced during the hydration reaction was shown to induce cell proliferation.

Omar et al (2005)[33] compared mineral trioxide aggregate (MTA) with calcium hydroxide $Ca(OH)_2$ clinically and radiographically as a pulpotomy agent in immature permanent teeth. Fifteen children, each with at least 2 immature permanent teeth requiring pulpotomy, were selected for this study. All selected teeth were evenly divided into 2 test groups. In group 1, the conventional Ca(OH)2 pulpotomy (control) was performed, whereas in group 2, the MTA pulpotomy was done. The children were recalled for clinical and radiographic evaluations after 3,6, and 12 months. Results showed that the follow – up evaluations revealed failure due to pain and swelling detected at 6 and 12 months postoperative evaluations in only 2 teeth treated with Ca(OH)2 and 4 teeth treated with MTA. Mineral trioxide aggregate showed clinical and radiographic success as a pulpotomy agent in immature permanent teeth (apexogenesis) and seems to be a suitable alternative to calcium hydroxide.

Kahtani et al (2005)[23] evaluated the seal created by varying depths of mineral trioxide aggregate (MTA) plugs placed in an orthograde

fashion in five groups of 10 teeth. One group received a 2mm thick orthograde apical plug of MTA, the second group a 5mm apical MTA plug, and the third group a 2mm apical MTA plug with a second 2mm increment, 24 h later. Results showed a statistically significant difference in only the 5mm apical plug, which completely prevented bacterial leakage.

***Santos et al (2005)*[38]** reported on Ca^{2+} release, pH and electrical conductivity of an experimental cement and compared them with those of MTA angelus. Five samples of each cement were prepared using plastic tubes 1mm is diameter and 10mm long. Each sample was sealed in a test tube containing 10ml deionized water which was analysed after, 24, 48, 72, 96, 192, 240, & 360 hrs for PH, electrical conductivity and calcium release. The concentration of calcium is obtained through atomic absorption spectroscopy. The experimental cement released calcium and hydroxy ions comparable with comparably with those released by MTA angelus. After 24 months the calcium ion release by EC was greater than MTA and two cements released the ions up to 360h storage in aqueous solution.

Asgary et al (2005)[4] conducted the study to analyse and compare the elemental constituents of white MTA and GMTA. Each cements was mixed with distilled water according to manufacturers instruction and placed in the cavities of depth 3 mm with appropriate condenser. The samples were immersed in saline and allowed to set in incubated at 37 degree c for 48 hrs. The elemental composition was determined using SEM equipped with light element energy dispersive spectrometer. The results showed that WMTA had smoother mixture, GMTA showed bigger crystals. The study concluded the observed concentration for Al2O3, MgO and particularly FeO in white MTA are considerably lower than those found in GMTA. The observed concentration of FeO in GMTA thought to be the primary response for variation in colour.

Camilleri et al (2005)[6] determined the constitution of two commercial versions of MTA and to analyse the surface morphology of the powder, and the set material under various conditions. The constitution of two commercial version of MTA was determined before and after mixing with water. The unset material was analysed using energy dispersive analysis by X-ray (EDAX) in a scanning electron microscope (SEM) and X-ray diffraction (XRD). The first

technique identified the constituent elements while XRD analysis identified the compounds or phases present. The set material was evaluated using EDAX. The surface morphology of the material stored under various conditions (100% humidity, immersion in water, or immersion in phosphate solution was evaluated using SEM. Results the EDAX showed the white MTA to the composed primarily of calcium silicon, bismuth and oxygen, with the gray MTA also having small peaks for iron and aluminium. The XRD analysis showed gray MTA to be composed primarily of tricalcium silicate and dicalcium silicate. The surface morphology of the materials differed under the various conditions, particularly following immersion in phosphate solution with crystal formation.

Hezaimi et al (2005)[21] assessed the sealing ability of orthograde MTA root canal filling against human saliva and to compare gray – coloured MTA and white – coloured MTA to vertically condensed guttapercha and sealer. Forty – three extracted single – rooted human teeth were serially instrumented to a file size 40 / 0.06 at the apex and obturated with either gray – coloured MTA (group A), white – coloured MTA (group B) , or guttapercha and Kerr Canal sealer EWT (group c). The teeth were then mounted in a model to test for saliva

leakage. Both MTA preparations were more resistant to human saliva leakage than vertically condensed guttapercha and sealer.

Camilleri et al (2006)[9] evaluated the suitability of fast setting cement formulations based on Portland cement as dental core build up materials using two different methods of testing compressive strength and evaluation of setting times. Four fast setting cements based on Portland cement were tested for setting time, constitution of cement and compressive strength. Ordinarily Portland cement was used as control. He concluded that all the fast setting cements included in this study set in <7 min and were not susceptible to changes in the compressive strength testing procedure at 1 and 7 days but at 28 days all the fast setting cements had a significantly higher strength.

Kogan et al (2006)[24] identified the types and amounts of additives required to achieve optimal settings properties of MTA for single visit clinical procedures. The effects of these additives on the compressive strength of the set MTA mixtures were also determined. Additives include saline 2% lidocane 3% sodium hypo chloride gel, chlorhexidine gluconate gel, K-y gelly and 5% calcium chloride. The settings time the evaluated using a Vicat apparatus, compressive

strength of there set materials were evaluated with an Instron machine. NaOcl gel, k-y gelly and 5% cacl2 decreased the settings time 20 to 25 minutes. Compressive strength of these set materials were significantly lower than MTA mixed with water. MTA mixed with NaOcl gel demonstrated good working properties and improved setting time, this combination may be viable in singly visit procedure where compressive strength of the material is not that important.

Walker et al (2006)[47] compared the flexural strength of MTA as a function of setting time and different hydration conditions 24 hrs and 72 hrs setting time with either one or two sided moisture explosive. MTA specimens were allowed to set for either 24 or 72 hrs was specimens exposed to moisture on either one or two specimen surfaces. There moisture conditions were used to simulate MTA setting with only external tissue moisture (one –sided moisture) versus tissue moisture in combination with a moisture intra canal cotton pellet (two sided moisture). Results showed that a moistened cotton pellet should be placed on the intra canal MTA surface under a temporary restoration and if possible, to optimize flexural strength, the moisture pellet should only remain in place for 24 hrs.

Islam et al (2006)[22] conducted study to use X-Ray diffraction to compare the major constituents present in ProRoot MTA, white MTA, ordinary portland and white port land cements. Specimens were prepared by packing dry powder into an X-ray holder which was placed on a flat glass slab. X-ray diffractometry of the 4 materials where carried out. The main constituents were found to be tri calcium silicate and tetra calcium alumino ferrite in all four cements with the additional presence of BI2O3 in ProRoot MTA and ProRoot MTA tooth coloured.

Sarkar et al (2006)[39] conducted a study to characterize the interaction of MTA with synthetic tissue fluid and compared it with neutral phosphate buffer saline solution in extracted human teeth. Samples were prepared in plastic vials using 0.25g of MTA and 1ml of distilled water, STF was added to each sample and stored in fluid incubator for 37 degree C. The canals were prepared and filled with MTA and exposed to STF 37 degree C. The precipitate and sectioned teeth were analysed using SEM, EDAX, ILP-AES AND XRD. The results showed that the precipitate matched hydroxy apatite (HA). MTA is contact using STF dissolves releasing all its major cationic

components and forms hydroxyapatite on the surface with the surrounding fluid.

Song et al (2006)[42] evaluated the chemical composition and crystalline structures of Portland cements, gray ProRoot MTA, white ProRoot MTA and gray MTA-angelus. X-ray diffraction analysis was used to identified and characterised crystalline phases and energy dispersive x-ray spectrometer was used to determine the chemical composition of the test material. Both powder form and set form were examined. Results showed that the crystalline structure and chemical composition of gray and white MTA were similar except for the presence of iron in gray MTA. Both were composed mainly of bismuth oxide and calcium silicate oxide. Portland cement was composed mainly of calcium silicate oxide and did not contain bismuth oxide. Gray MTA-angelus had a lower content of bismuth oxide than proRoot MTA. There were no noticeable difference in the chemical composition and crystalline structure between the powder and set forms of any of the material tested.

Danesh et al (2006)[16] compared the solubility, microhardness and radiopacity of proroot MTA with the portland cements type CEM1

and CEM2. Twelve ring molds were filled with the cements these samples were immersed in doubled distilled water for 1 minute, 10 minutes, 1 hour, 24 hour, 72 hours and 28 days mean loss of weight was determined. All the samples were tested according to the ISO standards. Results shown that after 28 days MTA had low solubility (0.78%) compared with CEM1 (31.38%) and CEM2 (32.33%). Microhardness of MTA was significantly higher compared with the two portland cements and was significantly more radio opaque. The study concluded that MTA displayed superior material property than the portland cement.

VanderWeele et al (2006)[46] evaluated the resistance to displacement of tooth-colored MTA when subjected to blood contamination and the use of different mixing liquids in a furcation perforation model. MTA was mixed with either sterile water, anesthetic solution, or saline. All samples underwent Instron testing at either 24 ,72 hours or 7days. The results showed that significantly greater force was required to displace all samples at 7 days than was required at 24 hours or 72 hours. He concluded that, allowing the MTA to set undisturbed for 7 days before placement of a coronal restoration may decrease the chances of MTA displacement.

Watts et al (2007)[48] tested the compressive strength, as a measure of relative set of WMTA and GMTA when mixed into sterile water (or) local anaesthesia and exposed to an acidic environment. Total of 248 samples of WMTA and GMTA were mixed into local anaesthetic agent or sterile water and placed in a phosphate buffered saline at PH 5.0 or 7.4 for a period of 7 or 28 days the specimen were tested using instron device. Results showed that when WMTA and GMTA mixed with local anaesthetic, WMTA was significantly stronger than GMTA, more time in PBS caused a significant decrease compressive strength. There was no consistent difference in compressive strength for WMTA and GMTA when mixed with sterile water.

Camilleri et al (2007)[10] reported the hydration mechanism of white MTA. The Chemical constitution of white MTA was study by viewing the powder in polished section under the scanning electron microscope (SEM), the hydration of both white MTA and white Portland cement (PC) was studied by characterizing cement hydrates viewed under the SEM, plotting atomic ratios, performing quantitative energy dispersive analyses with X-ray (EDAX) and by calculation of the amount of anhydrous clinker minerals using the

Bogue calculation. Results showed that Un-hydrated MTA was composed of impure tri-calcium and di-calcium silicate and bismuth oxide. The aluminate phase was scare. On hydration the white PC produced a dense structure made up of calcium silicate hydrate, calcium hydroxide, monosulphate and ettringite as the main hydration products. The un-reacted cement grain was coated with a layer of hydrated cement. In contrast MTA produced a porous structure was low. Bismuth oxide was present as un-reacted powder but also incorporated with the calcium silicate hydrate.

Nekoofar et al (2007)[32] examined the effect of condensation pressure on the surface hardness microstructure an d compressive strength of MTA. The material was mixed and packed into cylindrical polycarbonate tubes. Six groups of specimens were subjected to pressure of 0.06, 0.44, 1.68, 3.22, 4.46 and 8.88 MTA respectively. The microstructure were analysed using SEM after sectioning of the sample. Results showed higher condensation pressure produce lower surface hardness. A condensation pressure of 1.68 MTA conferred the maximum compressive strength. SEM showed samples with higher condensation shows few voids than prepared with lower condensation

pressure, reduction in crystalline formation due to lack of sufficient space for water molecules.

Namazikhah et al (2008)[31] evaluated the surface microhardness of mineral trioxide aggregate specimens following exposure of their surface to a range of acidic environment during hydration. The Material was mixed and packed into cylindrical polycarbonate tubes of 6 mm diameter and height of 12 mm. four groups of each ten specimens were formed using a pressure of 3.22MPA and expose to a PH 4.4, 5.4, 6.4, 7.4 respectively for 4 days. The results showed that more the acidic environment solution the more extensive the velocity of the specimen.

Camilleri et al (2008)[11] characterized the hydration products of white mineral trioxide aggregate. The cements were tested un-hydrated and after hydration and curing for 30 days at 37 degree C. Analysis of hydrated cement leachant was performed weekly inductively coupled plasma emission spectroscopy after which the cements were viewed under the scanning electron microscope to evaluate the current microstructure. Results showed MTA produced calcium silicate hydrate (C-S-H) and calcium hydroxide on hydration. MTA

produced a higher proportion of calcium ions on CH, a by-product of hydration and also by decomposition of C-S-H, the release of calcium ions reduces with time.

Camilleri et al (2008)[12] investigated the physical properties of a novel accelerated Portland cement. The setting time, compressive strength, pH and solubility of white Portland cement and accelerated Portland cement produced by excluding gypsum from the manufacturing process and adding bismuth oxide were evaluated. He concluded that the setting time of Portland cement can be reduced by excluding the gypsum during the last stage of the manufacturing process without affecting its other properties. Addition of bismuth oxide affected the properties of the novel cement.

Saghiri et al (2008)[36]evaluated the effect of ph on sealing ability of white effect mineral trioxide aggregate as a root end filling material. Root end filling were exposed to acidic environments with bph values 4.4,5.4,6.4,7.4 for 3days in the experimental groups. Microleakage was evaluated using bovine serum albumin. The earliest bovine serum albumin microleakage was observed in a ph value of 4.4 followed by ph values of 5.4,6.4&7.4 respectively

Camilleri et al (2009)[13] reviewed the chemical composition and hydration mechanism of Mineral trioxide aggregate (MTA). It is composed of Portland cement, with 4:1 addition of bismuth oxide added so that the material can be detected on a radiograph. The cement is made up of calcium, silicon and aluminium. The main constituent phases are tricalcium and dicalcium silicate and tricalcium aluminate. There are two commercial forms of MTA, namely the grey and the white. The difference between the grey and the white materials is the presence of iron in the grey material, which makes up the phase tetracalcium alumino-ferrite. This phase is absent in white MTA. Hydration of MTA occurs in two stages. The initial reaction between tricalcium aluminate and water in the presence of calcium sulphate results in the production of ettringite. Tricalcium and dicalcium silicate react with water to produce calcium silicate hydrate and calcium hydroxide, which is leached out of the cement with time.

Saghiri et al (2009)[37] evaluated morphologic microstructure and surface hardness of white mineral trioxide aggregate (WMTA) after exposure to a range of alkaline environments during hydration. WMTA was mixed and packed into 60 glass tubes. Four groups, each containing 15 tubes, were exposed to pH values of 7.4, 8.4, 9.4, and

10.4, respectively, for 3 days. In 12 tubes in each group, Vickers surface hardness was measured after exposure to alkaline environments. Three specimens in each group were prepared to be evaluated under a scanning electron microscope using scattered electron (SE) and backscattered electron (BSE) detectors. They found that the mean surface hardness values after exposure to pH values of 7.4, 8.4, 9.4, and 10.4 were 58.28, 68.84, 67.32 and 59.22, respectively. The difference between these values was statistically significant ($p = 0.000$). The SE detector revealed needle-shaped crystals at pH values of 7.4 and 8.4 and an amorphous microstructure at pH values of 9.4 and 10.4 on WMTA surface. The BSE detector showed more unhydrated structure and pores at pH values of 7.4 and 10.4 compared with pH values of 8.4 and 9.4.They concluded that the surface hardness can be influenced by different alkaline pH values. More porosity and unhydrated structure are observed in WMTA exposed to pH values of 7.4 and 10.4.

Materials & Methodology

MATERIALS USED

1. GMTA (ANGELUS, BRAZIL)
2. WMTA (ANGELUS, BRAZIL)
3. AN INDIGENOUS EXPERIMENTAL CEMENT - BIOCEM
4. n Butyric acid (S D FINE – CHEM LIMITED, INDIA)
5. Sodium butyrate (SISCO RESEARCH LABORATORIES PVT LTD, INDIA)
6. Sterile cotton
7. Sterile gauze
8. Distilled water
9. Silicon carbide-based sandpapers (WET OR DRY TM, 3 M; ST PAUL, MN, USA)

ARMAMENTARIUM

1. Sterile glass vials
2. Glass slab
3. Cement spatula
4. Sterile plastic container
5. Sterile pipette

6. Sterile Burette
7. Magnetic stirrer
8. Polyvinyl chloride (PVC) straw
9. Sterile amalgam condenser
10. Sterile amalgam carrier
11. Sterile scissors
12. Sterile disposable syringe

EQUIPMENTS

1. pH meter (COMPANY – INTECH, MODEL NO.1-10)
2. Custom-made device with stainless steel piston
3. Scanning Electron Microscope (COMPANY – PHILIPS XL 30 SEM, NETHERLANDS)
4. Optical microscope with Vicker's Microhardness Testing Machine (CLEMEX CMT.HD)

METHODOLOGY :

99 cylindrical moulds of size 6/4mm length fabricated using pvc pipe as mould and cold sterilized using 2 % glutaraldehyde.

↓

72 moulds were divided into 3 groups for Microhardness testing and 27 moulds were divided into 3 groups for SEM evaluation.

↓

Group 1-WMTA, Group 2- GMTA and Group 3-BIOCEM was mixed and condensed upto 5mm inside the prepared cylindrical moulds

↓

Each group was further divided into four sub-groups of various pH – a - 5.4 , b - 6.4 and c - 7.4

↓

The material was compacted evenly into the mould using a custom made device under a pressure of 3.22Mpa which was applied for one minute.

↓

A wet cotton pledget was placed onto the samples within the polyvinyl tube.

↓

The samples were stored at room temperature (20°C) with in a glass vial.

↓

The bottom of each vial contained gauze soaked in butyric acid buffered at pH 5.4, 6.4 and 7.4.

↓

The opening of the glass vial were covered by moist gauze and covered to ensure the presence of sufficient humidity inside the vials.

↓

After four days the samples were removed from the moulds and subjected to micro hardness testing and SEM evaluation.

RESULTS

The microhardness testing experiment results of WMTA, GMTA and BIOCEM at various pH are presented in Table-1.

It was found that the surface hardness values of all the experimental samples present at various pH were almost same.

Within the GMTA,WMTA and BIOCEM groups, the highest mean surface hardness values were observed following exposure to a pH 7.4. The values decreased following exposure to pH 6.4 and the lowest mean surface hardness values were observed following exposure to pH 5.4.

SCANNING ELECTRON MICROSCOPY:

The internal microstructure of all the specimens that were exposed to various pH revealed a variety of structures. Specimens exposed to butyric acid with ph 7.4 showed bundles of jagged needle like formations and had distinctive crystalline structure embedded within a more uniform and homogenous matrix that was partially covered by a gel form structure.

METHODOLOGY

PREPARATION OF THE MOULD

Ninety nine cylindrical moulds of size 6mm diameter and 10mm length were fabricated using sections of sterilized poly vinyl chloride pipe as mould. All the moulds were cold sterilized using 2% Glutaraldehyde for 6hrs, after which they were cleansed with sterile water and allowed to dry in open air.

MANIPULATION OF MTA

WMTA, GMTA and BioCem cement were mixed separately on a glass slab with a cement spatula in a water powder ratio as recommended by the manufacturer. The mixed cement was carried with amalgam carrier and was condensed upto 5 mm inside the prepared moulds using the amalgam condenser.

SAMPLE GROUP

Group 1 – GREY MTA

Group 2 – WHITE MTA

Group 3 – Experimental cement,BIOCEM

SUBGROUPS

a – pH 5.4

b – pH 6.4

c - pH 7.4

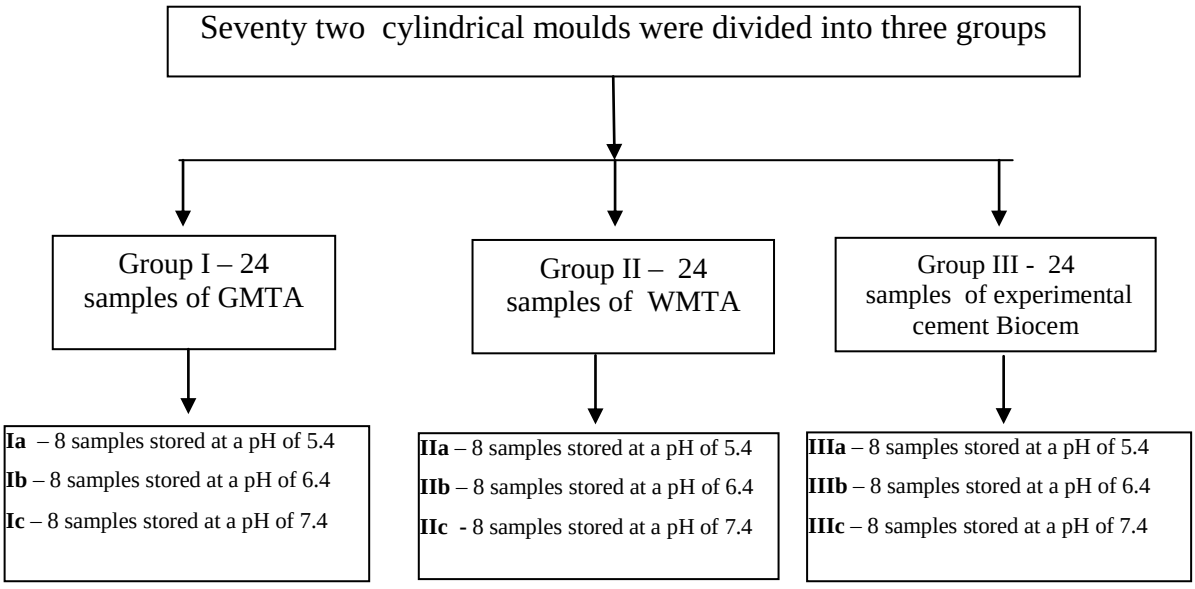
DIVISION OF THE SAMPLES

Total = 99 samples

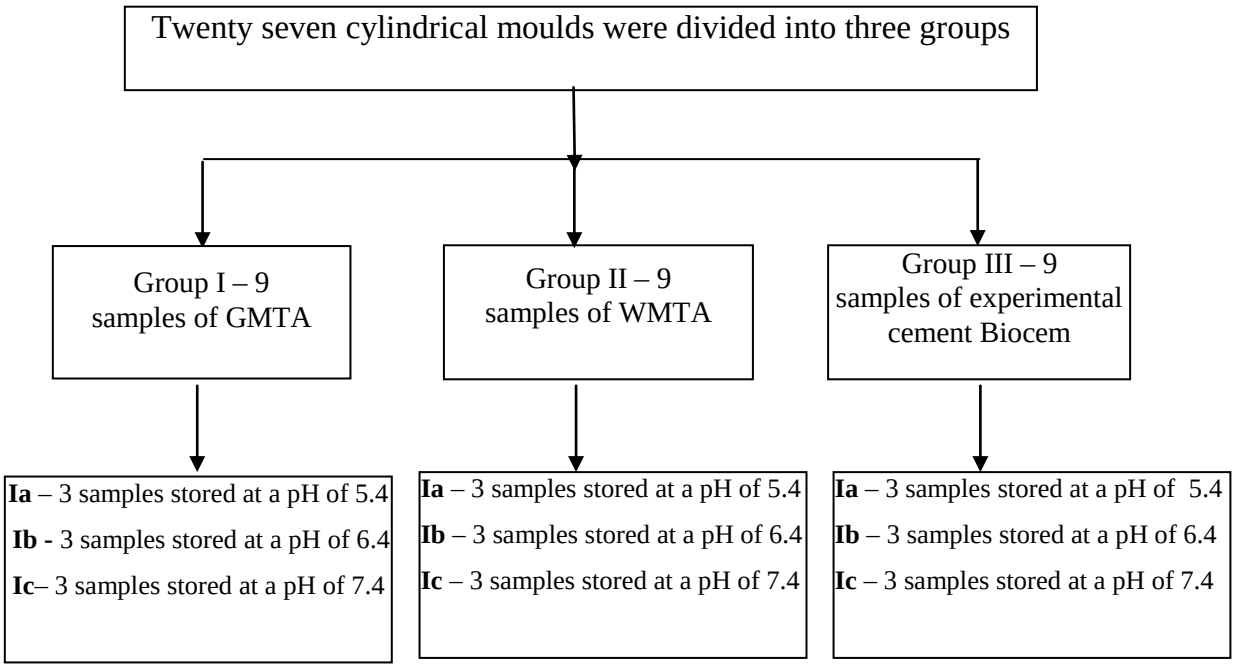
72 - for Microhardness

27 - for SEM

DIVISION OF THE SAMPLES FOR MICROHARDNESS TESTING



DIVISION OF SAMPLES FOR SEM EVALUATION



The material was compacted evenly into the cylindrical mould using a custom-made device containing a stainless steel piston with the similar internal diameter of polyvinyl cylindrical tubes under a pressure of 3.22Mpa which was applied for 1 min to standardise the compaction of material.

A wet cotton pellet was placed onto the samples within the polyvinyl tube to simulate the clinical scenario and stored at room temperature (20^0 C) within a glass vial for 4 days.

The bottom of each vial contained a piece of 2 cm X 2 cm gauze that had been soaked in butyric acid buffered at pH 5.4, 6.4 or

7.4 respectively. The control group contained gauze piece that had been soaked in distilled water.

Based on pilot experimentation, the acid-soaked pieces of gauze were replaced with fresh acid-soaked gauze every 24 h to ensure a consistent pH during the experimental period.

The openings of the glass vials were then covered by moist gauze and covered to ensure the presence of sufficient humidity inside the vials.

After 4 days, the MTA specimens were removed from the moulds.

PREPARATION OF BUTYRIC ACID AT VARIOUS pH.

Preparation of the salt solution

5 grams of sodium butyrate salt was mixed with 250 ml of distilled water. The mixing is done in a vibrating machine with a magnetic stirrer.

Preparation of butyric acid at various pH

1ml of butyric acid was pipetted out and diluted in 50 ml of distilled water. The diluted acid was added drop by drop into the prepared sodium butyrate solution with the help of a pre - adjusted burette.

A **pH meter** is an electronic instrument used to measure the pH (acidity or alkalinity) of a liquid. A typical pH meter consists of a special measuring probe (a glass electrode) connected to an electronic meter that measures and displays the pH reading.

The electrode present in the pH meter was placed inside the sodium butyrate salt solution and the circuit was closed. The readings were displayed in the pH meter. The required experimental pH was obtained by adding the acid in drops, to the salt solution. When the required pH was obtained, the addition of acid was stopped and the solution was stored in the sterile plastic container.

MICROHARDNESS TESTING

The surfaces exposed to acid on each specimen were then wet polished at room temperature using minimum hand pressure and silicon carbide-based sandpapers of varying particle size of 600-grit and 1200-grit.

The polished specimens were cleaned gently under light pressure distilled water to remove surface debris. To prevent dissolution or water sorption, the surfaces were dried gently by air spray.

The Vickers microhardness test of each specimen was performed with a square-based pyramid shaped diamond indenter at a full load of 50 g for 5 s at room temperature . Five indentations were made on the polished surface of each specimen at separated locations no closer than 1 mm to adjacent indentations or the specimen periphery.

The diagonal of the resulting indention was measured immediately under the microscope and the Vickers microhardness value displayed on the digital readout of the microhardness tester.

The Vickers microhardness (HV) was calculated based on the following formula

$$HV = \frac{2F\sin\frac{136^\circ}{2}}{d^2} \qquad HV = 1.854\frac{F}{d^2}$$

approximately where F = load/kg; and d = the mean of the two diagonals of the impression made by the indenter in millimetres.

The mean value of the hardness value obtained was calculated to determine the hardness value for each specimen.

SCANNING ELECTRON MICROSCOPY:

For the microstructural morphological evaluations by SEM, twelve specimens (three for each group) were prepared using the same pressure to condense the material and then stored for 4 days under the same conditions whilst exposed to either pH 5.4, 6.4 and 7.4, respectively.

The specimens were sectioned into two halves using a disposable surgical scalpel blade No. 15 to initiate the crack and were further split into two halves using a cutter.

The surfaces were sputter-coated with gold using a Polaron Sputter Coater and specimens were analysed with Scanning Electron Microscope.

The micrograph images from the SEM analysis showing the qualitative internal micro structure of the set MTA were evaluated at the same depth within the specimens in terms of the presence of micro channels and type of crystal formation.

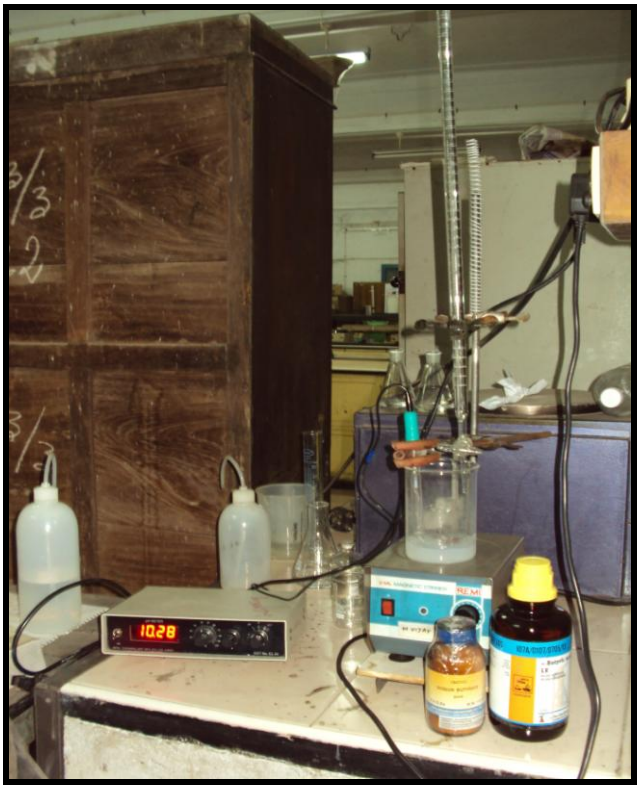
Determination of the constituent elements in Biocem (EDAX analysis):

Energy Dispersive Analysis by X- ray was performed under the SEM.A thin layer of powder was dispersed over carbon double sided tape attached to an aluminium stub. The stubs were carbon coated for electrical conductivity. The specimens were then viewed under the SEM and X-ray analysis was carried out to determine the constituent elements of the powder.

ARMAMENTARIUM

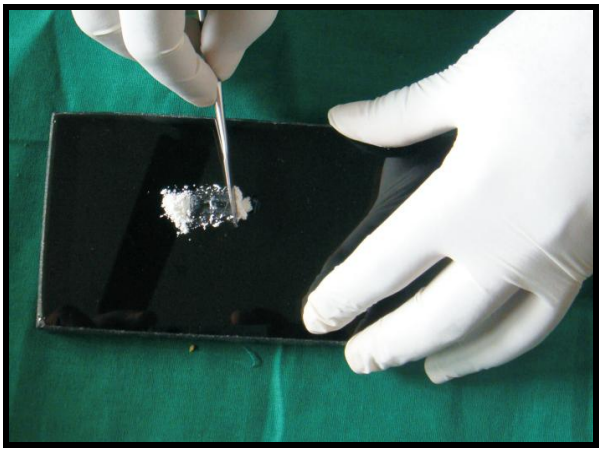
CUSTOM - MADE DEVICE

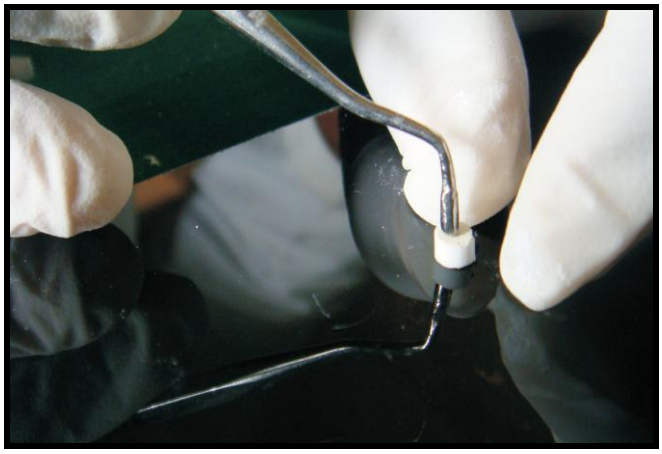
pH METER



BUTYRIC ACID SOLUTION AT VARIOUS pH

MANIPULATION OF MTA

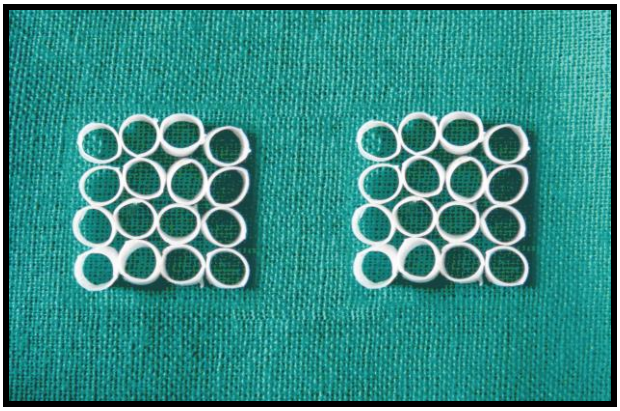




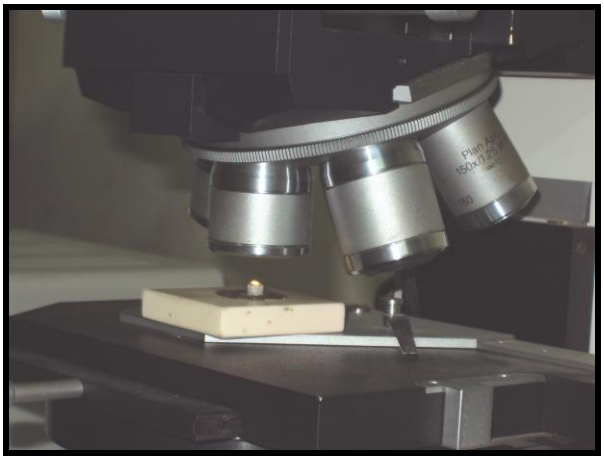
WET COTTON PLEDGET PLACED ON THE MTA SAMPLES

SAMPLES STORED WITHIN THE GLASS VIALS

CYLINDRICAL MOULDS



VICKERS MICROHARDNESS TESTING MACHINE WITH OPTICAL MICROSCOPE



GOLD SPUTTER COATING

SCANNING ELECTRON MICROSCOPE

Results

As the ph decreased to 6.4 structures such as microchannels, porosities and assymetrical crystalline formations in the form of laminated cross stratified structures were seen. The porosity increased and the pore size diameter was larger when compared to that of ph 7.4

Specimens exposed to more acidic ph ie, 5.4 revealed porosities, depressions caused by air bubbles and also a few microchannels. The porosity was extensive on the surface of MTA. The pore size diameter was still larger when compared to ph 6.4 and 7.4.

EDAX analysis:

The Elemental composition of the new experimental cement, Biocem was studied using EDAX analysis. It was found that Biocem contained 42% of Ca, 32% of O, 10% of C, 8% of Bi, 5% of Si, 2% of Al, 1% of K.

TABLE-1 MICROHARDNESS EXPERIMENTAL RESULTS OF GMTA, WMTA, BIOCEM:

Group I Grey MTA (HV) (Vicker's Hardness)			**Group II White Mta (HV)**			**Group III BIO-Cem (HV)**		
A) pH 5.4	**B) pH 6.4**	**C) pH 7.4**	**A) pH 5.4**	**B) pH 6.4**	**C) pH 7.4**	**B) pH 5.4**	**C) pH 6.4**	**D) pH 7.4**
38.6	43.9	58.5	37.1	44.4	56.8	39.3	49.6	53.9
37.9	42.6	58.5	38.7	48.7	54.0	38.6	44.7	58.1
39.1	46.8	53.9	39.2	46.9	52.5	37.2	46.9	56.2
36.5	45.7	54.3	40.6	47.5	56.8	40.2	47.2	55.1
40.1	47.7	51.9	36.4	49.5	56.1	42.1	46.2	52.1
40.4	43.5	52.3	42.2	47.7	54.2	37.9	44.6	52.7
39.2	46.3	56.8	37.9	46.3	56.3	36.8	48.1	54.5
38.3	44.7	57.4	35.6	46.6	52.6	41.4	45.8	53.6

STATISTICAL ANALYSIS

The results of the present study were subjected to statistical analysis to interpret the significant differences between the various pH in each group and also between the groups**.** One-Way ANOVA followed by Tukey HSD test was used for statistical analysis in the present study.

One-way Analysis Of Variance (ANOVA) was used to study the overall variance within and between groups. It is the extension of the between groups t-test to a situation in which more than two groups are compared simultaneously. However, it is not possible to identify the differences between the various subgroups with the help of the P values obtained from ANOVA. Therefore a specific statistical test was used for intra- group comparison.

Tukey HSD (Honestly significant difference) test is a nonparametric multiple comparison test. It is a post hoc test designed to perform a pair wise comparison of the means to identify the specific subgroups in which significant differential expression occurs. The term ‘comparisons’ typically refers to comparisons of two groups. ‘Multiple comparisons’ enters when there are several such comparisons. Hence, Tukey HSD is done in order to determine which groups differ from each other.

No statistically significant difference were observed in all the three experimental groups of WMTA, GMTA, and BIOCEM at all pH levels (Table- 2).

The difference between the values of GMTA specimens exposed to different PH (5.4, 6.4, 7.4) were statistically significant (Table- 3).

The difference between the values of WMTA specimens exposed to different PH (5.4, 6.4, 7.4) were statistically significant (Table- 4).

The difference between the values of BIOCEM specimens exposed to different PH (5.4, 6.4, 7.4) were statistically significant (Table- 5).

Table-2: One-Way ANOVA followed by Tukey HSD for inter group comparison between GMTA, WMTA, BIOCEM.

MATERIALS	**PH 5.4**		**PH 6.4**		**PH 7.4**	
	MEAN	**SD**	**MEAN**	**SD**	**MEAN**	**SD**
GMTA	38.76	1.25	45.15	1.77	55.45	2.69
WMTA	38.46	2.20	47.20	1.56	54.91	1.81
BIOCEM	39.23	1.96	46.64	1.69	54.53	1.94
P VALUE	> 0.05		> 0.05		> 0.05	

Note *P value >0.05 denotes non significance*

pH 5.4

Group	Group	Mean Difference	Std. Error	P value
Grey MTA	White MTA	.3000	.9219	.943
	Bio-Cem	-.4625	.9219	.871
White MTA	Grey MTA	-.3000	.9219	.943
	Bio-Cem	-.7625	.9219	.691
Bio-Cem	Grey MTA	.4625	.9219	.871
	White MTA	.7625	.9219	.691

Note *P value >0.05 denotes non significance*

pH 6.4

Group	Group	Mean Difference	Std. Error	P value
Grey MTA	White MTA	-2.0500	.8375	.058
	Bio-Cem	-1.4875	.8375	.202
White MTA	Grey MTA	2.0500	.8375	.058
	Bio-Cem	.5625	.8375	.782
Bio-Cem	Grey MTA	1.4875	.8375	.202
	White MTA	-.5625	.8375	.782

Note *P value >0.05 denotes non significance*

pH 7.4

Group	Group	Mean Difference	Std. Error	P value
Grey MTA	White MTA	.5375	1.0901	.875
	Bio-Cem	.9250	1.0901	.678
White MTA	Grey MTA	-.5375	1.0901	.875
	Bio-Cem	.3875	1.0901	.933
Bio-Cem	Grey MTA	-.9250	1.0901	.678
	White MTA	-.3875	1.0901	.933

Note *P value >0.05 denotes non significance*

TABLE 3: ONE–WAY ANOVA FOLLOWED BY TUKEY HSD TEST FOR COMPARISON WITHIN THE GMTA GROUP

pH	MICROHARDNESS	
	MEAN	SD
5.4	38.76[a]	1.25
6.4	45.15[b]	1.77
7.4	55.45[c]	2.69
P VALUE	< 0.001**	

Note *** denotes significant at 1% level.*

Different alphabets between subgroups denote significance at 5% level.

TABLE-4 : ONE–WAY ANOVA FOLLOWED BY TUKEY HSD TEST FOR COMPARISON WITHIN THE WMTA GROUP

pH	MICROHARDNESS	
	MEAN	**SD**
5.4	38.46[a]	2.20
6.4	47.20[b]	1.56
7.4	54.91[c]	1.81
P VALUE	< 0.001**	

Note *** denotes significant at 1% level.*

Different alphabets between subgroups denote significance at 5% level.

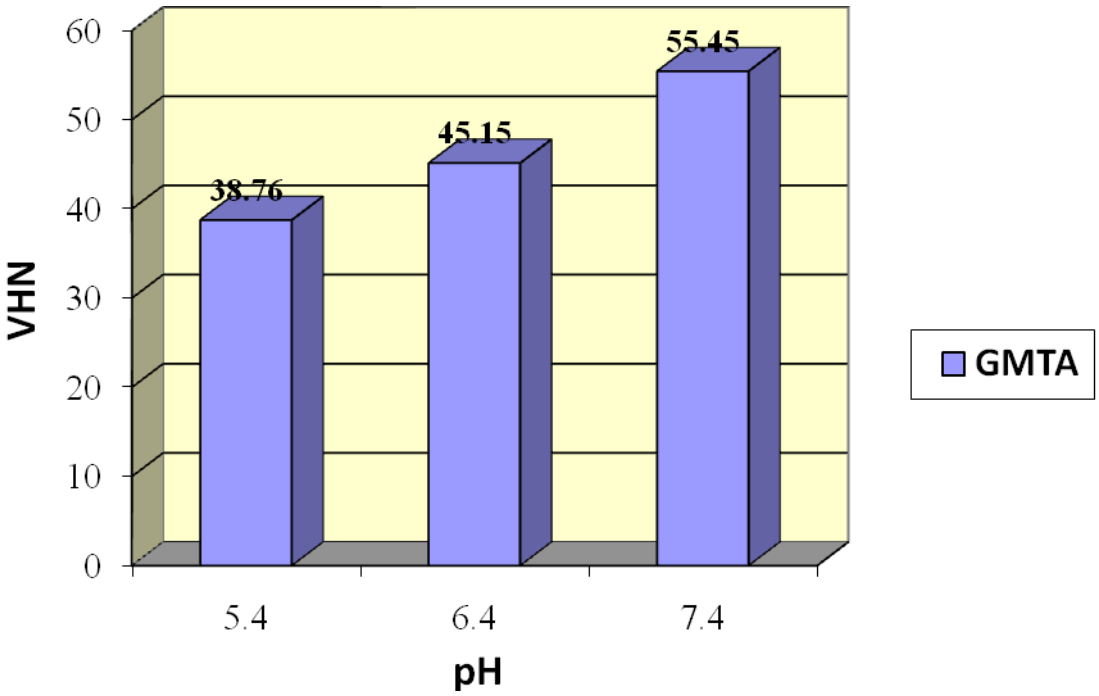
TABLE-5 : ONE–WAY ANOVA FOLLOWED BY TUKEY HSD TEST FOR COMPARISON WITHIN THE BIOCEM GROUP

pH	MICROHARDNESS	
	MEAN	**SD**
5.4	39.23[a]	1.96
6.4	46.64[b]	1.69
7.4	54.53[c]	1.94
P VALUE	< 0.001*	

Note *** denotes significant at 1% level.*

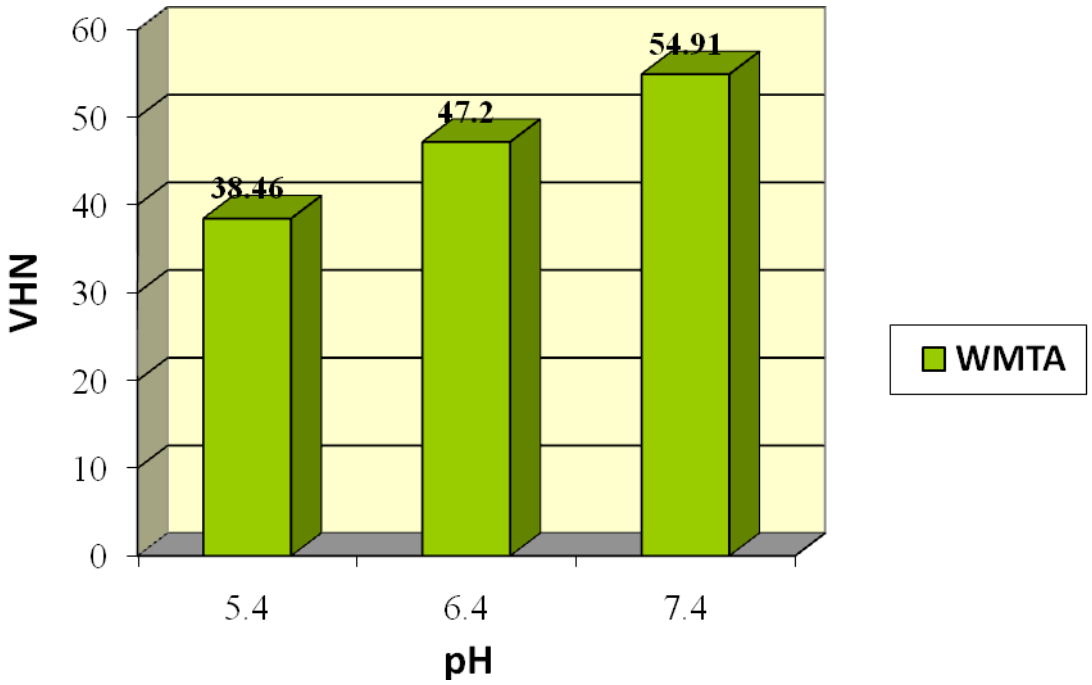
Different alphabets between subgroups denote significance at 5% level.

COMPARISON OF MICROHARDNESS BETWEEN pH 5.4, 6.4,&7.4 WITHIN THE GMTA GROUP



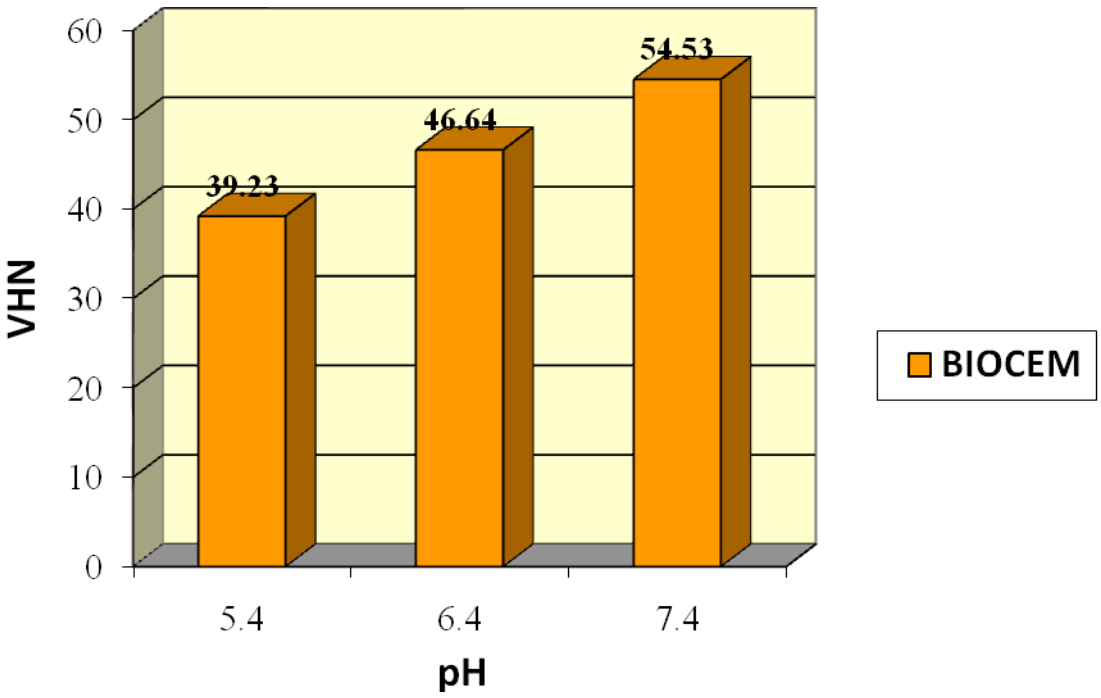
VHN = Vicker's Microhardness

COMPARISON OF MICROHARDNESS BETWEEN pH 4.4, 5.4, 6.4,&7.4 WITHIN THE WMTA GROUP



VHN = Vicker's Microhardness

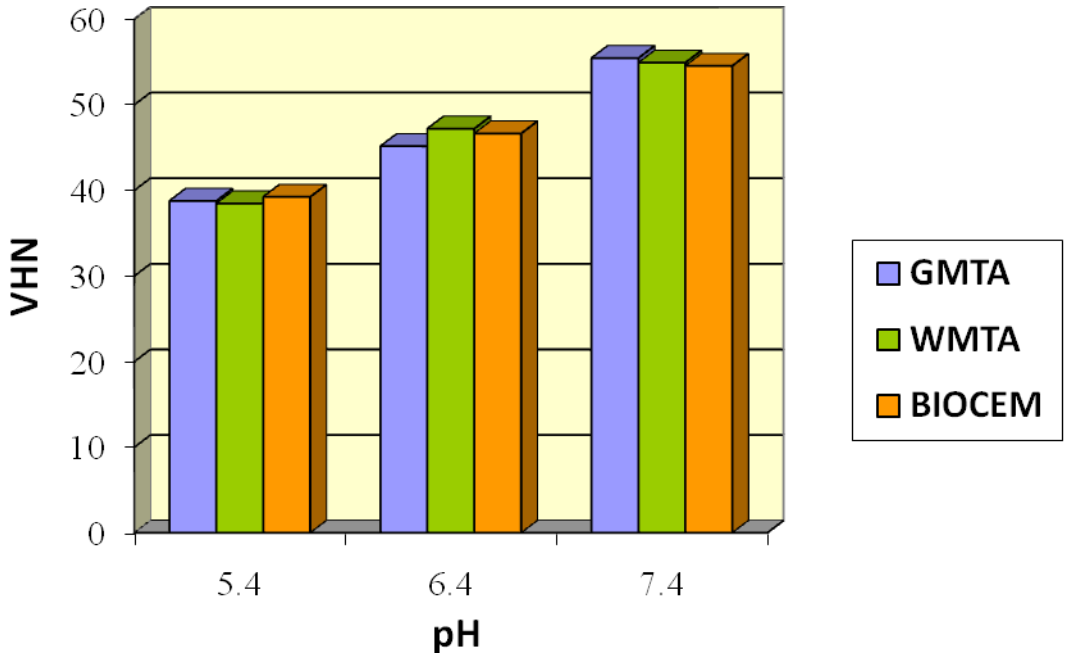
COMPARISON OF MICROHARDNESS BETWEEN pH 4.4, 5.4, 6.4,&7.4 WITHIN THE BIOCEM GROUP



VHN = Vicker's Microhardness

COMPARISON OF MICROHARDNESS BETWEEN GMTA,

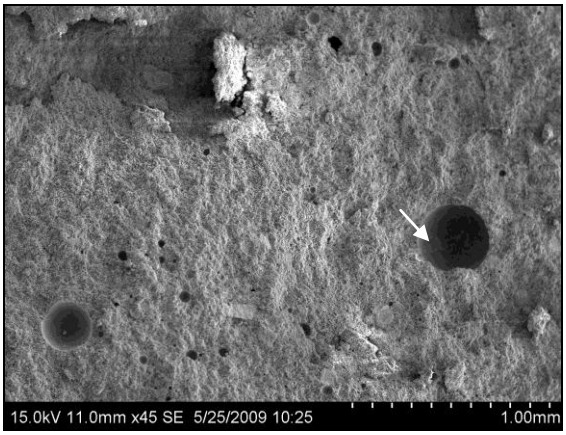
WMTA, BIOCEM AT pH 4.4, 5.4, 6.4,&7.4.



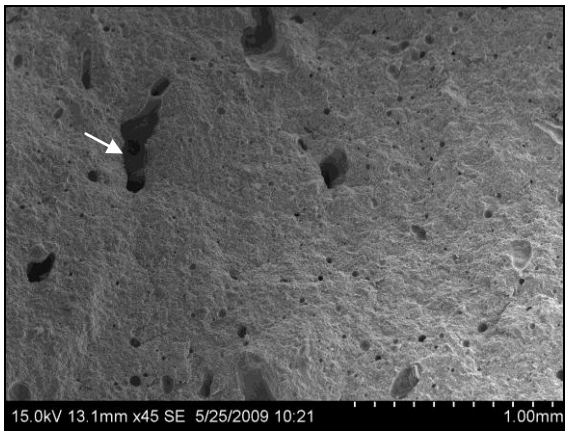
HV = Vicker's Microhardness

SEM EVALUATION OF GREY MTA EXPOSED TO VARIOUS pH

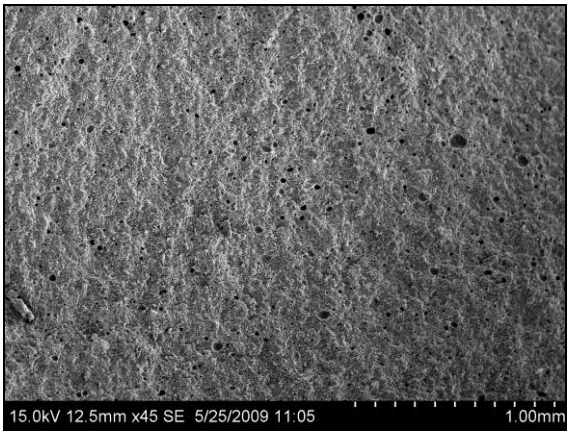
pH 5.4 (Fig. a)



pH 6.4 (Fig b)

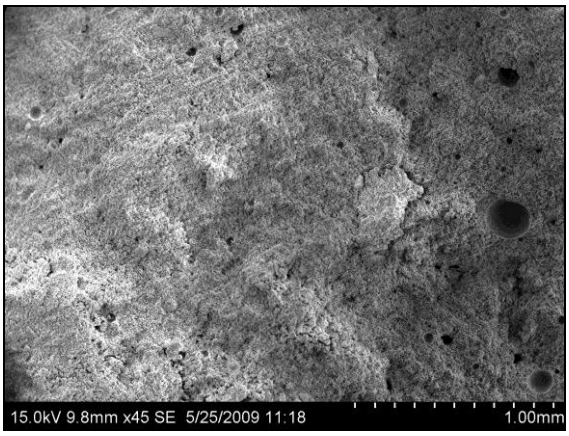


pH 7.4 (Fig c)

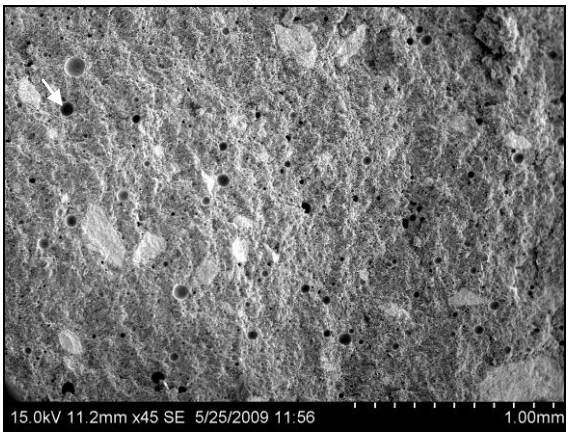


SEM EVALUATION OF WHITE MTA EXPOSED TO VARIOUS pH

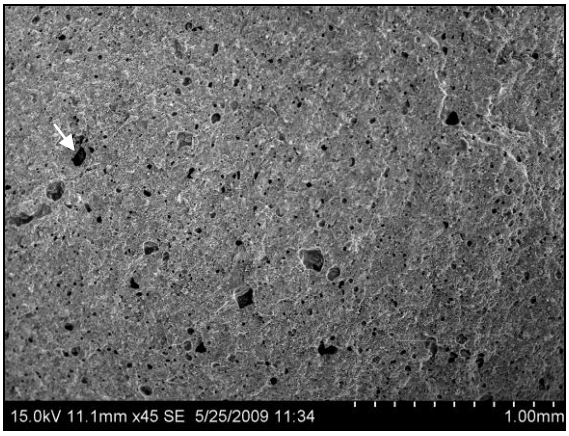
pH 5.4 (Fig d)



pH 6.4 (Fig e)

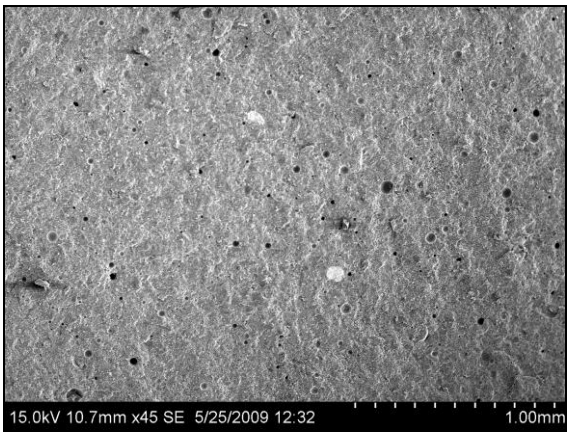


pH 7.4 (Fig f)

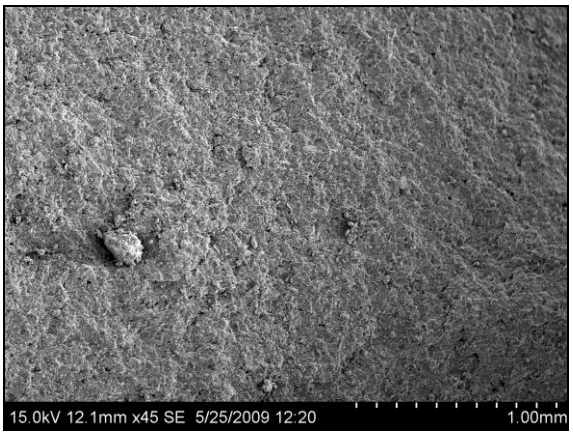


SEM EVALUATION OF BIOCEM EXPOSED TO VARIOUS pH

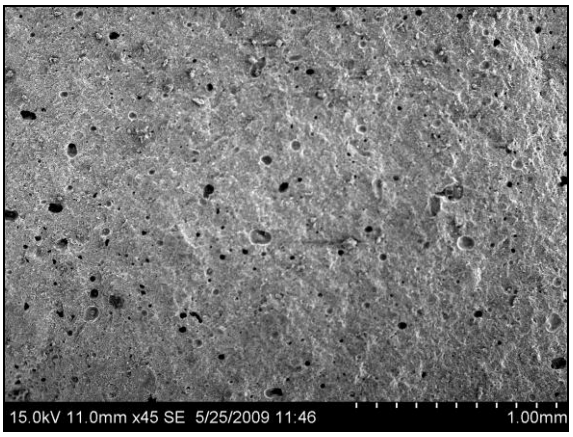
pH 5.4 (Fig g)



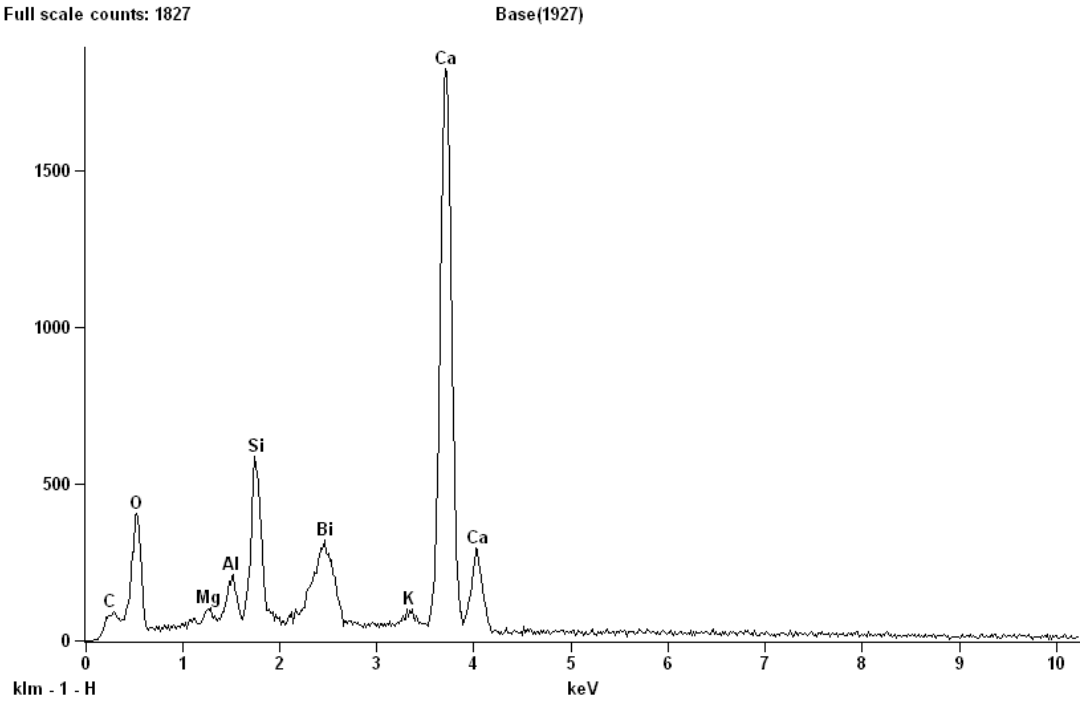
pH 6.4 (Fig h)



pH 7.4 (Fig i)



EDAX ANALYSIS FOR BIOCEM



Live Time: 100.0 sec.

Acc.Voltage: 15.0 kV Take Off Angle: 35.0 deg.

QUANTITATIVE RESULTS

Element	*Net Counts*	*Net Counts Error*	*Weight %*	*Atom %*	*Formula*	*Compnd %*
C	852	+/- 116	9.96	19.80	C	9.96
O	3802	+/- 190	32.08	47.87	O	32.08
Mg	298	+/- 104	0.30	0.29	Mg	0.30
Al	1392	+/- 260	1.31	1.16	Al	1.31
Si	6028	+/- 322	5.22	4.44	Si	5.22
K	649	+/- 244	0.84	0.51	K	0.84
K	0	+/- 404	---	---		---
Ca	27075	+/- 542	41.90	24.96	Ca	41.90
Ca	0	+/- 248	---	---		---
Bi	4887	+/- 482	8.38	0.96	Bi	8.38
Total			100.00	100.00		100.00

Discussion

DISCUSSION

Root end surgery has historically served as the most conservative final effort to resolve peri-radicular inflammation after the non-surgical root canal therapy has failed or not possible.[48] The procedure usually consists of root-end exposure and resection, as well as preparation of a class 1 cavity and placement of a root- end filling material.[44] Ideally this procedure should exclude bacteria and their byproducts from the periapical tissues, allowing regeneration or repair of these tissues to take place. It should also help in the formation of new cementum layer, covering the root surface. Thus the main objective of root- end filling material is to provide an apical seal that inhibits the migration of antigen from the root canal system into the periradicular tissues.[23]

Almost every available dental restorative material or cement has at one time or another been suggested for use as a root- end filling material. However, the commonly used root- end filling materials which are supported by the dental literature are MTA , two zinc oxide and eugenol - based cements - Intermediate restorative material

(IRM) and Super EBA. MTA was shown to be superior to other commonly used root-end filling materials in studies of marginal adaptation and leakage. Many studies revealed MTA to be an excellent biocompatible material. Several reports have also indicated that MTA has an antimicrobial effect.[7]

At present, Mineral trioxide aggregate (MTA) is widely used in endodontic therapy. This biomaterial was developed at Loma Linda university in the year 1993, originally used for the purpose of root end filling. Over time its clinical application has expanded to vital pulp therapy, including pulpotomy, apexification, surgical and non-surgical perforation repair.[38] The use of MTA as a root-end filling material was identified due to the fact that the material is a hydraulic cement i.e. it sets in the presence of water. A dental cement that sets and develops its properties in the presence of moisture is highly desirable.[9]

Apexification procedures using calcium hydroxide have been historically used to induce apical closure. Anderson et al showed that calcium hydroxide when placed within the root canals of immatured teeth over one year, showed a fifty percent reduction in strength of

the root dentin versus the controls. In contrast most of the studies revealed MTA to be the best material for apexification. In addition to its good sealing ability and bio compatibility, MTA was found to facilitate the over growth of cementum, regeneration of the periodontal ligament and formation of bone.[48]

Mineral trioxide aggregate (MTA) consist of 50-75% wt calcium and 15-25% silicon dioxide. These two components together comprise 70-95% of the cement. When these raw materials are blended they produce tricalcium silicate, dicalcium silicate, dicalcium alumina and tetra calcium alumino ferrite which on addition of water hydrates to form silicate hydrate gel. Torabinejad et al. developed the original product (gray MTA). The main constituents of this material were calcium silicate ($CaSi0_4$), bismuth oxide (Bi_20_3), calcium carbonate ($CaC0_3$), calcium sulphate (CaS04), and calcium aluminate *($CaAl_20_4$)*. MTA was originally marketed as gray coloured preparation and has been associated with occasional staining of the teeth. Therefore a white MTA material has been recently developed to overcome this concern. There is a limited research comparing the properties and clinical application of white MTA and a few have

shown a competitive analysis between white and Gray MTA. Tetra calcium alumino ferrite is reportedly removed in the Gray MTA formula so as to provide a hue which matches more closely to that of the teeth. Therefore it seems reasonable to suspect that the absence of significant FeO in WMTA is most likely to cause the change in color from Gray to White.[3]

BIOCEM, an experimental indigenous MTA like cement which is composed of a mixture of calcium oxide, silicates and bismuth oxide has also been tested in this study because of its similarity to WMTA. The Elemental composition of the new experimental cement, Biocem was studied using EDAX analysis. It was found that Biocem contained 42% of Ca, 32% of O, 10% of C, 8% of Bi, 5% of Si, 2% of Al, 1% of K. This experimental cement did not show the presence of any toxic heavy metals and it was also arsenic free.

Within the human body under normal physiologic conditions, any minor change in pH is controlled by the ***carbonic acid-bicarbonate buffer system*** and the other pH regulatory systems active in connective tissue; periodontal tissue is no exception. However, in

certain clinical applications, MTA is placed in an environment where inflammation is present and the surface of the unset material will be exposed to a low pH environment (acidic pH). In addition, in some clinical situations like open apex, nonvital teeth with periapical lesions , lateral or furcal perforations with radiolucent lesions, MTA might be directly exposed to an acidic environment.[31]

The pH of the human abscess has been measured as low as 5.0. This low pH could potentially inhibit not only the setting reaction, but also affect adhesion and increase the solubility of dental materials.[48] Physical and chemical properties of MTA also might be influenced in a low pH environment. Impeded MTA setting as well as reduced strength and hardness has been reported in an acidic environment.[37] Thus it is possible that variations in the pH value of the host tissues, because of pre-existing pathologic conditions at the time of MTA placement, might affect its hardness and jeopardize the outcome of the treatment.[31,37] Hence, in the present study, the influence of pH on the surface hardness and microstructure of GMTA, WMTA and BIOCEM has been investigated and compared.

Poly Vinyl chloride moulds were used to compact the WMTA and GMTA and BIOCEM, as these moulds do not interfere with the chemical constituents of the material. The moulds were of size 10mm length and 6mm in diameter, as 3-5mm of MTA is the ideal thickness needed to prevent microleakage.

Mixing was done according to manufacturer's instructions and the material was compacted evenly into the cylindrical mould using a custom-made device containing a stainless steel piston with 5mm internal diameter of polyvinyl cylindrical tubes. A study by Nekoofar et al reported that condensation pressure may affect the strength and hardness of MTA. According to them, higher the condensation pressure, lower the surface hardness. Based on the inference of their study, the condensation pressure of 3.22Mpa was applied for 1 min. The samples were thus subjected to a constant vertical force that was translated into a transverse and equally distributed pressure that compacted the MTA evenly into the cylindrical mould.[32]

Hydraulic cements are finely ground materials that when mixed with water gradually or instantly set and harden either in air or

water. The reaction results in the formation of hydrated compounds whose strength increases with time. The hydration of MTA has been reported to consist of two separate reactions. The initial reaction was between tricalcium aluminate and water, which, in the presence of gypsum found in small quantities in MTA, resulted in the production of ettringite, which later formed monosulphate, once the gypsum was depleted. The low levels of alumina reported in MTA affected the production of ettringite and monosulphate, usually formed on hydration of Portland cement. The main reaction between the tricalcium and dicalcium silicate and water resulted in the production of calcium silicate hydrate gel, which is poorly crystalline, and calcium hydroxide. Set MTA was composed of numerous residual un-hydrated cement grains, which had a dense rim of hydration product, made up of pure calcium silicate hydrate. There was very little ettringite or monosulphate present. Un-reacted bismuth oxide particles and calcium hydroxide were also detected. The calcium silicate hydrate had taken up bismuth, which replaced the silica in the calcium silicate hydrate structure.

Bismuth oxide, added to enhance the radio-opacity of MTA, was reported to be present only in 8.4% level in set MTA, as against the 21.6% in the unset material. The bismuth formed a part of the structure of the calcium silicate hydrate gel and also affected the precipitation of calcium hydroxide in the hydrated paste. Both bismuth and calcium were leached out from MTA. The calcium leached out decreased over a five-week period, while the bismuth oxide levels increased.

The production of calcium hydroxide by MTA would explain the similar mode of tissue reaction to MTA and calcium hydroxide reported previously. It has been reported that MTA released calcium ions and promoted an alkaline pH. MTA has been shown to leach calcium ions several days after the initiation of hydration and setting of the material. These calcium ions diffuse through the defects in the dentin in root canals filled with MTA, and the concentration increases with time. When in contact with tissue fluid, an amorphous calcium phosphate phase initially formed, which later transformed to an apatite phase, with the latter consisting of calcium-deficient, poorly crystalline, B-type carbonated apatite

crystallites. Amorphous calcium phosphate is a key intermediate that precedes biological apatite formation in skeletal calcification.[13]

MTA is a type of hydraulic cement that can set in the presence of water.[16] **Matt G et al, Sarkar et al,** showed that additional moisture from a cotton pellet is crucial for the material to establish its optimum properties. Hence, a wet cotton pellet was placed over the samples within the polyvinyl tube to provide relative humidity for setting reaction of the material.[28,39]

Mineral trioxide aggregate has been shown to release soluble fractions of calcium hydroxide in both the short and long-term sufficient to maintain the pH of the surrounding environment at a high level. Duarte et al confirmed that MTA released calcium ions as a result of hydration of calcium oxide, the main component of MTA and Portland cement.[17]

Torabinejad et al reported the pH value of MTA to be between 10.5 and 12.9. The biological properties of MTA, e.g. the ability to induce changes in cellular activity of osteoblasts, have been attributed to its alkalinity.[43] Santos et al noted that the pH of MTA samples increased to a peak of 10.39 within the first 24 hours after

mixing followed by a decrease to 7.72 within 360 hours. It is recommended that MTA be allowed to set untouched for 72 hours or longer to decrease the chance of MTA displacement.[38]

Vander Weele et al showed that significantly greater forces was required to displace all samples at 7 days than was required at 24 hours and 72 hours. This suggests that after initial 24 hours or 72 hours disturbance, MTA was still setting. It was concluded that allowing the MTA to set undisturbed for 7 days before placement of a coronal restoration may decrease the chances of MTA displacement.[46] When inflamed tissues are removed during periapical surgery, the pH of tissues adjacent to root- end filling and its interface with dentin might be changed to neutral in less than 3 days. Furthermore, **Lee et al** demonstrated that, in situations where the initiating and perpetuating factors of an inflammatory process are removed by appropriate treatment, it is possible that the pH of the environment returns to normal in a shorter time period than the 7 days. Therefore in this study, MTA was exposed for no longer than 4 days to the acidic solution in order to simulate the situation in which the initiating and perpetuating factors of inflammatory processes are removed by appropriate treatment.[25]

Lee et al studied the effect of pH on the hydration process of MTA. They immersed and stored MTA samples in solutions of pH 5, 7 and 7.4 for 7 days and reported that their microhardness at low pH was reduced. However, immersion of the material in acid does not simulate clinical conditions, as most often only a part of the surface of the MTA will be exposed to an acidic environment. Hence in the present study, attempts were made to mimic the clinical situation by exposing MTA to acid - soaked pieces of gauze placed at the bottom of each vial.[26]

Various types of acid have dissimilar effects on the physical and chemical characteristics of MTA. The type of acid used by Lee et al in his study was not stated. Lota et al demonstrated that considerable changes in the microstructure of hydrated cement occurred in the presence of polyacrylic acid when compared with a control paste. Rai et al reported that hydration of Portland cement was considerably retarded when malic acid was added. In the presence of tartaric acid, the silicate hydration-phase of Portland cement was retarded strongly. In contrast, Singh et al revealed that lactic acid accelerated the hydration of Portland cement by increasing the

crystalline character of calcium hydroxide resulting in advanced growth of the hydration products.

Different concentrations of citric acid have been shown to have dissimilar effects on Portland cement. Singh et al indicated that 0.1% citric acid accelerated the hydration process of Portland cement whereas concentrations > 0.1% retarded hydration.

In the present study butyric acid, a by-product of anaerobic bacterial metabolism was used to simulate the clinical conditions of periradicular infections. The butyric acid used was buffered with sodium butyrate to pH 5.4 (n = 10), 6.4 (n = 10), and 7.4 (n = 10).[31,35]

To ensure a consistent pH during the experimental period, the acid-soaked pieces of gauze were replaced with fresh acid-soaked gauze every 24 hours. The openings of the glass vials were then covered by moist gauze and covered to ensure the presence of sufficient humidity inside the vials. Similar procedures were observed in previous studies by Namazikhah et al , Nekoofar et al, & Saghiri et al.[31,32,35,36]

MTA fails to set on few occasions, and this has been reported in a few studies. In those cases the MTA material required replacement at a further appointment. One reason for this lack of hydration during setting reaction may be attributed to the acidic pH of the inflamed tissue in contact with the material, including the presence of various acids secreted by bacteria in the infected site. The present study supports the results given by Lee et al that, MTA does not harden well as the pH decreases. Further, a few more studies revealed that, the physical and chemical properties of MTA might be influenced in a low pH environment.[26]

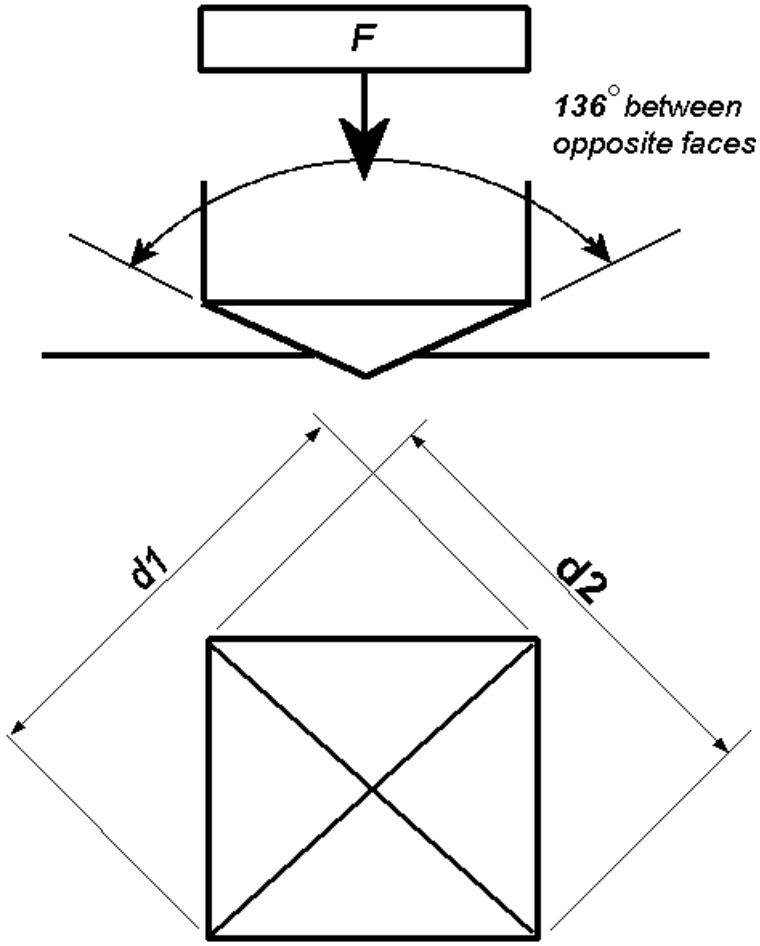
The microhardness of a material is not a measure of a single property. It is influenced substantially by other fundamental properties of the material such as yield strength, tensile strength, modulus of elasticity and crystal structure stability. Thus, it can be used as an indicator of the setting process and the overall strength or resistance to deformation when compared with baseline information. It can also indicate the effect of various setting conditions on the overall strength of a material.

Strength and hardness may not be of paramount importance when MTA is used as a root-end filling, but any subsequent replacement of an associated orthograde root canal filling would require the root-end filling to have adequate strength as reported by Saghiri et al. The applications for the use of MTA have broadened and sufficient strength must be considered important. The extended use poses additional demands on the development of MTA. The strength and hardness of the material must be viewed as a more critical property. The term microhardness test usually refers to static indentations made with loads not exceeding 1 kilogram force (kgf).

There are two universal types of microhardness test - Vickers and Knoop. Both are based on the ability of the surface of a material to resist penetration by a point under a specified load. The main difference is attributed to the shape of the diamond indenter. The shape of the Vickers diamond indenter is a square pyramid whereas the shape of the Knoop diamond indenter is an elongated pyramid shape. Measurement of the Vickers microhardness formed the basis of the present investigation. The surface being tested generally requires a metallographic finish; the smaller the load used, the higher the surface finish required.

Precision microscope was used to measure the indentations and in this study. An optical microscope (CLEMEX, CMT), with a magnification of X 500 was used to measure to an accuracy of ±0.5 micrometres. Also with the same observer differences of ±0.2 micrometres can usually be resolved. It should, however, be added that considerable care and experience are necessary to obtain this accuracy.

The Vickers microhardness test method consists of indenting the test material with a diamond indenter, in the form of a right pyramid with a square base and an angle of 136 degrees between opposite faces subjected to loads not exceeding 1 kgf (figure below). The full load is normally applied for 10 to 15 seconds. The two diagonals of the indentation left in the surface of the material after removal of the load are measured using a microscope and their average calculated. The area of the sloping surface of the indentation is calculated. The Vickers microhardness is the quotient obtained by dividing the kgf load by the square mm area of indentation.



Vickers Pyramid Diamond Indenter Indentation

The Vickers Diamond Pyramid microhardness number is the applied load (kgf) divided by the surface area of the indentation (mm^2)

$$HV = \frac{2F\sin\frac{136^o}{2}}{d^2} \qquad HV = 1.854\frac{F}{d^2} \text{ approximately}$$

Where:

F= Load in kgf,

d = Arithmetic mean of the two diagonals, *d1* and *d2* in mm

VHN = Vickers microhardness number

When the mean diagonal of the indentation has been determined the Vickers microhardness may be calculated from the above formula. The advantages of the Vickers microhardness test are that extremely accurate readings can be taken, and just one type of indenter is used for all types of metals and surface treatments.

Vickers MicroHardness Number Calculator

Force (kgf)		Enter value
Mean diagonal length d (mm)		Enter value
VHN		Result

Danesh et al. reported that the Vickers microhardness of MTA was 39.99. Lee et al. noted that the microhardness of MTA using the Knoop scale was 51.20.[31]

One-Way ANOVA followed by Tukey HSD test was used for statistical analysis in the present study. One-way Analysis Of Variance (ANOVA) is used to study the overall variance within and between groups. However, it is not possible to identify the differences between the various subgroups with the help of the P values obtained from ANOVA.

Tukey HSD (Honestly significant difference) test is a nonparametric multiple comparison test and a specific statistical test used for intra- group comparison.. It is a post hoc test designed to perform a pair wise comparison of the means to identify the specific subgroups in which significant differential expression occurs. Hence, Tukey HSD was done in this study for inter group comparison.

The results of the present study were tabulated and subjected to statistical analysis to interpret the significant differences between the various pH in each group and also between the groups**.** The results of the present study revealed that there was no statistical significant difference observed between all the three experimental groups of WMTA, GMTA, and BIOCEM at all pH levels. However, Vickers microhardness of all the experimental samples were significantly

affected by low pH environments. At pH 7.4, the surface microhardness of GMTA was 55.45, WMTA was 54.91, BIOCEM was 54.53 with the Vickers scale, this value decreased significantly following exposure to pH 6.4 whereas at pH 5.4 the microhardness values of GMTA was 38.76, WMTA was 38.46 and BIOCEM was 39.23. This finding of the present study was in accordance with **Lee et al** who reported that weaker specimens resulted from immersion and storage in a low pH environment.[26]

Saghiri et al in their microleakage study reported that the time needed for leakage to occur was significantly longer in samples stored at higher pH values. In their study root – end fillings were exposed to acidic environments at different pH values of 4.4, 5.4, 6.4 and 7.4 for 3 days. They buffered butyric acid at the above pH levels to simulate the acidic environment. In that study microleakage was evaluated by using bovine serum albumin and the earliest microleakage was observed in a pH value of 4.4 followed by pH values 5.4, 6.4, and 7.4, respectively.[36] The microhardness and the microstructure of all the experimental samples in the present study were significantly affected by the low pH environment (i.e) the microhardness at pH 5.4 was least followed by pH 6.4 and 7.4. Thus

it was observed that the results of Saghiri et al's study coincided with the results of this present study.

In contrast **Roy et al**, reported that an acid environment alone does not hinder but also enhances the sealing ability of MTA. In their study MTA was used with Calcium phosphate cement (CPC) matrix, and samples were exposed to a pH value of 5 for 24 hours but the type of acid used in their study was not stated. Chow et al demonstrated CPC liberates water during setting, thereby not allowing MTA to be exposed directly to the acidic environment. Moreover Taylor et al showed that various types of acid have led to different physical and chemical effects on Portland cement. The differed result of the above study might be attributed to the usage of CPC matrix, unbuffered acidic solutions, short term exposure time to acidic solution (24 hours) ,use of dye penetration method and the type of acid used in the study.[35]

In the present study CPC matrix has not been used and the experimental samples were directly in contact with the acidic environment. Hence the results of the present study differed from the results of the study by Roy et al.

Presence of acidic pH environment, may not only impede the MTA setting, but also reduce its strength and hardness, increase its solubility and further it can affect its adhesion property. A study revealed that mixing MTA with an acidic solution like 2% lidocaine hydrochloride with an epinephrine concentration of 1: 100000 reduced the compressive strength of MTA in an acidic environment. MTA specimens were stored in various pH solutions for 7 days and the mean Knoop microhardness values of MTA specimens were reported.[31]

They indicated that specimens stored at pH 5 were weaker than those stored at higher pH. However, in clinical situations MTA might be exposed to an acidic environment, in the periradicular tissues where active inflammation is present.

Watts et al studied the effects of pH and mixing agents on the temporal setting of tooth- colored and gray MTA. He tested the compressive strength, as a measure of relative set cement, of WMTA and GMTA when mixed with sterile water or local anaesthetic and exposed to an acidic environment. He concluded that an acidic pH caused a decrease in compressive strength (and thus possibly the

quality of the set cement) in WMTA and GMTA when mixed with local anesthetic solution. The reason for the decrease in strength was due to the presence of the acidic environment that has deleterious effects on the setting characteristics of MTA. On the contrary, when the specimens were mixed with sterile water, they revealed an obvious increase in the compressive strength values.[48] In the present study, to simulate the acidic environment, buffered butyric acid was used at various pH levels. The results of the present study was also in accordance with the results of the study by Watts et al.

Scanning electron microscope was used to analyse the surface topography of the experimental groups .The surfaces of the samples were sputter-coated with gold for electrical conduction using a polaran sputter coater and specimens were analysed with an EBT1(electron beam technology). In the present study, SEM was chosen to evaluate the microstructural and morphological features of all the experimental samples.

In the SEM analysis, the internal microstructure of all the specimens that were exposed to various pH revealed a variety of structures such as microchannels, depressions caused by air bubbles

(Fig a and b), assymetrical crystalline formations in the form of laminated cross stratified structures, bundles of jagged needle like formations, homogenous matrix that was partially covered by a gel form structure (Fig c, f and i). However, specimens exposed to butyric acid with pH 7.4(neutral pH) had distinctive crystalline structure embedded within a more uniform matrix partially covered by colloidal gel. On the other hand, greater degree of porosity was observed in all the experimental samples that were exposed to the low pH environments (Fig a, d and g) ; although it was not possible to grade precisely and objectively the degree of porosity within the SEM examination.

Utilizing scanning electron microscope, Lee et al evaluated how various physiological environments affect the hydration behavior and physical properties of mineral trioxide aggregate (MTA). The samples were hydrated in distilled water, normal saline, pH 7, and pH 5. They found that the microstructure of hydrated MTA consists of cubic and needle-like crystals.[26] In the present study, specimens exposed to pH 7.4 also showed distinctive crystalline structure embedded within a more uniform matrix. Bundles of needle-like crystals were also appreciated. The surface changes in the present

study when exposed to pH 7.4 was similar to the results of the study by Lee et el. Similarly in his study, in those specimens immersed in pH 5, he observed only erosion of cubic crystal surfaces instead of needle like crystals. SEM micrographs of MTA stored in pH 5 clearly showed that the crystalline structure maturity paled in contrast to the formations of the other specimens. Hence, they concluded that, an acidic environment of pH 5 adversely affected both the physical properties and the hydration behaviour of MTA. In the present study, greater degree of porosity was seen in all the experimental samples that were exposed to the pH 5.4 and pH 6.4. No needle- like crystals were observed when exposed to low pH environments. This result of the present study was in accordance with the results of the study by Lee et al.[38]

Recent studies are focussed to evaluate the influence of alkaline environment on the microstructure and microhardness of MTA. Stefopoules et al showed that calcium hydroxide pretreatment adversely affect the WMTA's sealing ability leading to the assumption that calcium hydroxide interferes with WMTA at the apical region. The marginal adaptation and sealing ability of the apical barrier were tested by means of a dye tracer (basic fuchsine)

after longitudinal sectioning. They showed that the residual calcium hydroxide might be a mechanical or chemical obstacle to MTA's adaption to the root canal walls and thereby affecting its sealing ability.[37]

MTA has been advocated for use in the closure of open apex. The treatment procedure in open apex teeth include, the placement of calcium hydroxide paste as an inter appointment intra canal medicament to eliminate or reduce bacterial contamination before placement of MTA. After the placement of calcium hydroxide in the root canal, the pH value of dentin adjacent to calcium hydroxide change to range of 11.1 to 12.2(alkaline pH).

Chemical interaction between calcium hydroxide and MTA might influence the surface characteristics of MTA. It has also been reported that increasing calcium hydroxide concentration might increase chemical shrinkage of Portland cement. Such shrinkage can cause changes in microhardness and SEM images of WMTA.

Current literature reveals that studies are focussed to evaluate the effect of different alkaline pH values on surface hardness of WMTA as an indicator of the setting process during hydration. They concluded that surface hardness can be influenced by different

alkaline pH values. [37] The present study was limited to investigate the microhardness and microstructure of MTA in acidic pH, and compared it with neutral pH. The sample preparation methodology and standardization varies for alkaline environment. Therefore, further studies have to be conducted to investigate the influence of alkaline pH on the microhardness and microstructure of MTA to enhance its physical and chemical properties.

The present study also investigated the microhardness and the microstructure of the indigenous experimental cement - Biocem under acidic and neutral conditions. EDAX analysis of the same sample was carried out and it revealed that it was free of arsenic. The suitability of Biocem for clinical applications should be determined by subjecting the material to various levels of biocompatibility tests to prove its "Bioacceptance" and followed by FDA Approval.

Summary

SUMMARY

The present study was designed to evaluate the surface microhardness of white and grey MTA following exposure to a range of acidic and neutral environments during hydration. In addition, BIOCEM an indigenous MTA like material, has also been studied. EDAX analysis was performed to determine the constituent elements in Biocem.

The morphological and microstructural features of the samples were studied using SEM. 99 cylindrical moulds of size 6mm diameter and 10mm length were fabricated and was used in this study. In this seventy two cylindrical moulds were used for microhardness testing and twenty seven cylindrical moulds were used for SEM evaluation.

The samples were divided into three groups, Group I – containing 24 samples of GMTA, Group II – 24 samples of WMTA and Group III - 24 samples of BioCem. The three groups were further divided into three sub - groups at pH 5.4, pH 6.4, pH 7.4. Similar grouping was done for SEM evaluation.

WMTA, GMTA and BIOCEM was mixed and condensed upto 5mm inside the prepared cylindrical moulds. The material was compacted evenly into the mould using a custom made device under a pressure of 3.22Mpa which was applied for one minute. A wet cotton pellet was placed onto the samples within the polyvinyl tube. The samples were stored at room temperature (20°C) with in a glass vial for 4 days. The bottom of each vial contained gauze soaked in butyric acid buffered at pH 5.4, 6.4 and 7.4.The opening of the glass vial were covered by moist gauze and covered to ensure the presence of sufficient humidity inside the vials.

After four days the samples were removed from the moulds and subjected to microhardness testing and SEM evaluation. The results of the present study were subjected to statistical analysis to interpret the influence of various pH in each group and also between the groups**.** One-Way ANOVA followed by Tukey HSD test was used for statistical analysis in the present study.

Conclusion

CONCLUSION

Within the limitations of this in vitro study, it was concluded that,

1. The surface hardness of all the experimental MTA groups and Biocem were impaired when exposed to acidic environment.

2. The greatest surface hardness values were observed following exposure to pH 7.4(neutral pH) in all the experimental samples.

3. The other interesting findings were that microhardness values decreased following exposure to pH 6.4 and pH 5.4. However there were no statistical difference between the GMTA and WMTA groups and also with the experimental cement Biocem, at the above pH levels.

4. The internal microstructure of all the specimens exposed to butyric acid with pH 7.4 had distinctive crystalline structure embedded within a more uniform matrix partially covered by colloidal gel. As the pH decreased to 6.4, the porosity

increased and the pore size diameter was larger when compared to that of 7.4.

5. Specimens exposed to a more acidic environment (pH 5.4) revealed extensive porosity on the surface of the experimental samples. The pore size diameter of the specimens exposed to pH 5.4 was still larger when compared to the specimens exposed to pH 6.4 and pH 7.4.

In the new horizon, the advancement in the technological field of biocompatibility testing can predict the property and design the material that can elicit customized biological responses.

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